The Effects of Eupatilin (Stillen®) on Motility of Human Lower Gastrointestinal Tracts

Seung-Bum Ryoo1, Heung-Kwon Oh1, Sung A Yu1,2, Sang Hui Moon1, Eun Kyung Choe1,3, Tae Young Oh4, and Kyu Joo Park1

1Division of Colorectal Surgery, Department of Surgery, 2Department of Physiology, Seoul National University College of Medicine, Seoul 110-744, 3Healthcare Research Institute, Seoul National University Hospital Healthcare System Gangnam Center, Seoul 135-984, 4Dong-A Pharmaceutical Co, Seoul 130-823, Korea

Gastrointestinal motility consists of phasic slow-wave contractions and the migrating motor complex (MMC). Eupatilin (Stillen®) has been widely used to treat gastritis and peptic ulcers, and various cytokines and neuropeptides are thought to be involved, which can affect gastrointestinal motility. We performed a study to identify the effects of eupatilin on lower gastrointestinal motility with electromechanical recordings of smooth muscles in the human ileum and colon. Ileum and colon samples were obtained from patients undergoing bowel resection. The tissues were immediately stored in oxygenated Krebs-Ringer's bicarbonate solution, and conventional microelectrode recordings from muscle cells and tension recordings from muscle strips and ileal or colonic segments were performed. Eupatilin was perfused into the tissue chamber, and changes in membrane potentials and contractions were measured. Hyperpolarization of resting membrane potential (RMP) was observed after administration of eupatilin. The amplitude, AUC, and frequency of tension recordings from circular and longitudinal smooth muscle strips and bowel segments of the ileum and colon were significantly decreased after admission of eupatilin. Eupatilin elicited dose-dependent decreases during segmental tension recordings. In conclusion, eupatilin (Stillen®) showed inhibitory effects on the human ileum and colon. We propose that this drug may be useful for treating diseases that increase bowel motility, but further studies are necessary.

Key Words: Colon, Eupatilin, Ileum, Motility, Smooth muscle

INTRODUCTION

Gastrointestinal motility consists of phasic slow-wave contractions and the migrating motor complex (MMC) in various animal models. Slow waves originate from electronic membrane potential changes in interstitial cells of Cajal (ICC) and generate spontaneous and rhythmic phasic contractions. The enteric nervous system affects the generation and migration of the MMC, which consists of periodic mass movements. The phasic contractions act to mix materials in the intestine and the MMC advances material from the oral to the anal end of the intestine [1-3]. Human gastrointestinal motility is thought to be similar, but more complex. For example, three-dimensional networks of ICCs or ICCs in line with the septa (ICC-SEP) might generate effective contractions of thick smooth muscles [4-7].

Disorders related to gastrointestinal motility might be caused by abnormalities of ICCs or of the enteric nervous system. There have been many efforts to develop a specific drug to treat diseases of increasing or decreasing gastrointestinal motility [8]. Irritable bowel syndrome (IBS) is the most common functional bowel disease, which frequently presents with abnormal bowel motility. Lifestyle modification is still the recommended treatment because effective drugs to improve dysmotility and relieve symptoms are not widely available [9-11].

Eupatilin (Stillen®) is an ethanol extract from an oriental herb, Artemisiaeasiatica Nakai. Eupatilin has already been widely used to treat gastritis and peptic ulcers. Eupatilin has anti-inflammatory and anti-oxidative cytoprotective effects against gastric mucosal damage and enhances regeneration of damaged mucosa [12,13]. Various cytokines and neuropeptides that can affect gastrointestinal motility are thought to be involved in the mechanism of action

ABBREVIATIONS: MMC, migrating motor complex; ICC, Interstitial cell of Cajal; IBS, irritable bowel syndrome; KRB, Krebs-Ringer solution; RMP, resting membrane potential; LM, longitudinal muscle; CM, circular muscle; AUC, area under the curve; PG, prostaglandins; NO, nitric oxide; HT, hydroxytryptamine; FDA, Food and Drug Administration.
METHODS

Tissue acquisition

Human ileum and colon samples were obtained immediately from operations for non-obstructive bowel diseases. There was no greater resection of the bowel than medically necessary because the amount of tissue needed for the experiments was very small. This study was approved by the Institutional Review Board of the Clinical Research Institute of the Seoul National University Hospital (IRB approval number: H-0603-071-170).

After resection of the bowel, 4×2 cm ileal or colonic segments were removed from the resected sections. Specimens were immediately placed into oxygenated Krebs-Ringer solution (KRB). The KRB contained (in mM) 120.4 NaCl, 5.9 KCl, 15.5 NaHCO3, 11.5 glucose, 1.2 MgCl2, 1.2 NaH2PO4 and 2.5 CaCl2. This solution had a pH of 7.3∼7.4 at 37.5°C when bubbled to equilibrium with 97% O2 and 3% CO2.

Tissue preparation

1. Cross-sectional preparation for intracellular recordings

Tissues were transferred to a Petri dish coated with Sylgard (Dow corning Co., USA) and pinned downed in a dissecting dish. The muscles were cut parallel to the longitudinal muscle (LM) fibers with a knife consisting of a pair of sharp parallel scalpel blades set 1.5 mm apart and turned on side to expose a cross section of all of the muscle layers in the electrophysiological chamber. The chamber was constantly perfused with prewarmed, preoxygenated KRB solution. The temperature was maintained at 37.5±0.5°C. The muscles were equilibrated for at least 2 hours before experiments began. Conventional microelectrode recordings were performed using sharp microelectrodes filled with 3 M KCl [6]. Membrane potentials were measured with a high-input resistance electrometer and outputs were displayed on an oscilloscope. Outputs were measured using pClamp software R (version 9.0. Axon Instruments, Foster City, CA, USA) and Origin Software (MicroCal Software, Northampton, MA, USA) programs. Resting membrane potential (RMP) (mV), wave and spike amplitude (mV), and the frequency (/min) of the slow waves were analyzed (Fig. 1A).

Smooth muscle strip preparation for tension recording

Ileal and colonic tissues were pinned down on a Petri dish coated with Sylgard with the mucosal side facing upward. The mucosal and submucosal layers were gently removed with a pair of scissors. Longitudinal and circular muscle (CM) bundles were obtained by sharp dissection. The size of the muscle strips were 2 mm in width and 1 cm in length.

For contractile activity recordings, the muscle strips were attached with a suture to an isometric strain gauge (World precision Instruments, Sarasota, FL, USA) in a tissue chamber perfused with pre-warmed, preoxygenated KRB solution. One end of the muscle strips was anchored to an isometric strain gauge and the other end was fixed to a steel bar [6]. The temperature was maintained at 37.5±0.5°C. The muscle strips were equilibrated for at least 2 hours before experiments began, and a resting force of 9.8 mN (1 g) was applied. The mechanical signals were digitized and recorded by Acknowledge software (Biopac Systems, Inc, Goleta, CA) for data analysis. Frequency (/min), amplitude (mN) and the area under the curve (AUC, sec×mN/min) of contraction waves were analyzed. In the muscle strip recordings, the area under the curve was defined as the integrated area under a single wave (Fig. 1B).

Preparations for tension recording of ileal or colonic segments

Ileal or colonic segments, with the mucosa and submucosa intact, were prepared by cutting the whole layer of a segment parallel to longitudinal muscle. The size of the segments was 4 cm in length and 2 cm in width. A
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**Results**

**Intracellular recording of membrane potentials**

Slow waves in ileum and colon tissues were detected by intracellular recording (Fig. 2). Administration of eupatilin significantly hyperpolarized the RMP at ileal smooth muscle cell at 1 μM (−57.67±16.53 vs −59.77±17.03, p=0.043). The hyperpolarization of RMP was also observed after administration of 2.5 (−65.63±12.16 vs −67.93±11.87, p=0.043) and 5 μM (−61.82±18.43 vs −63.03±18.60, p=0.043). However, the wave amplitude, spike amplitude and frequency were not significantly changed after administration of eupatilin, and electrical activity remained present. Eupatilin induced hyperpolarization of the RMP of colonic smooth muscle cell at 1 μM (−55.47±6.59 vs −57.50±5.78, p=0.008). The hyperpolarization of RMP was also induced at 2.5 (−57.98±7.75 vs −59.78±6.79, p=0.005) and 5 μM (−56.76±7.48 vs −61.02±7.89, p=0.018). The wave amplitude, spike amplitude and frequency were not changed. Detailed analyses of intracellular recordings of membrane potential are described in Table 1.

**Smooth muscle strip tension recordings**

Contractile waves were detected by smooth muscle strip tension recordings in CM and LM. The contractions of smooth muscle strips were reduced after administration of eupatilin in ileum and colon samples (Fig. 3). The amplitude was significantly decreased after administration of 1 μM of eupatilin in ileal CM (4.15±1.00 vs 2.26±1.05, p=0.043) and LM (6.52±2.66 vs 4.66±2.42, p=0.020). The AUC was also decreased at 1 μM of eupatilin in ileal CM (20.79±3.31 vs 10.32±4.63, p=0.043) and LM (32.46±10.20 vs 20.12±10.33, p=0.006). The frequency was decreased at 1 μM of eupatilin in ileal CM (3.45±1.23 vs 2.50±1.96, p=0.043) and LM (3.31±1.28 vs 2.48±1.43, p=0.042). The amplitude, AUC and frequency were also decreased significantly after administration of 2.5 and 5 μM of eupatilin in ileal CM and LM (p<0.050). Eupatilin significantly reduced the amplitude of colonic CM (17.35±3.97 vs 5.43±1.82, p=0.001) and LM (17.26±3.04 vs 6.56±4.41, p=0.001) at 1 μM. The AUC was also decreased at 1 μM of eupatilin in colonic CM (493.37±60.63 vs 238.13±140.12, p=0.001) and LM (619.23±183.64 vs 257.30±191.57, p=0.001). The frequency was decreased at 1 μM of eupatilin in colonic CM (1.38±0.08 vs 1.21±0.05, p=0.001) and LM (1.40±0.07 vs 1.22±0.09, p=0.001). The amplitude, AUC and frequency were also decreased significantly after administration of 2.5 and 5 μM of eupatilin in colonic CM and LM (p<0.050). Detailed analyses of the smooth muscle strip tension recordings are described in Table 2.
Table 1. Intracellular recordings of membrane potential changes before and after administration of eupatilin

| Segment | Concentration (μM) | Before | After | p-value |
|---------|-------------------|--------|-------|---------|
| Ileum   | 1 μM (n=5)        | RMP    | −57.67±16.53 | −59.77±17.03 | 0.043* |
|         |                   | Wave Amp | 9.52±5.09 | 9.24±4.34 | 0.500 |
|         |                   | Spike Amp | 27.41±6.46 | 27.00±1.86 | 0.893 |
|         |                   | Freq   | 13.80±15.53 | 8.91±8.38 | 0.080 |
|         | 2.5 μM (n=5)      | RMP    | −65.63±12.16 | −67.93±11.87 | 0.043* |
|         |                   | Wave Amp | 10.17±2.81 | 11.44±5.93 | 0.500 |
|         |                   | Spike Amp | 27.03±13.50 | 28.52±14.59 | 0.225 |
|         |                   | Freq   | 25.89±28.90 | 18.88±27.60 | 0.080 |
|         | 5 μM (n=5)        | RMP    | −61.82±18.43 | −63.03±18.60 | 0.043* |
|         |                   | Wave Amp | 8.87±7.34 | 9.30±6.70 | 0.686 |
|         |                   | Spike Amp | 25.04±11.60 | 24.85±15.47 | 0.500 |
|         |                   | Freq   | 21.15±28.21 | 22.51±32.78 | 0.500 |
| Colon   | 1 μM (n=9)        | RMP    | −55.47±6.59 | −57.50±5.78 | 0.008* |
|         |                   | Wave Amp | 7.93±5.24 | 8.24±5.51 | 0.515 |
|         |                   | Spike Amp | 26.58±21.73 | 28.78±17.14 | 0.767 |
|         |                   | Freq   | 19.42±15.55 | 15.20±11.53 | 0.674 |
|         | 2.5 μM (n=10)     | RMP    | −57.98±7.75 | −59.78±6.79 | 0.005* |
|         |                   | Wave Amp | 13.26±10.51 | 10.81±7.49 | 0.575 |
|         |                   | Spike Amp | 27.02±15.99 | 28.34±16.08 | 0.386 |
|         |                   | Freq   | 28.23±19.68 | 19.61±18.25 | 0.074 |
|         | 5 μM (n=7)        | RMP    | −56.76±7.48 | −61.02±7.89 | 0.018* |
|         |                   | Wave Amp | 12.16±5.37 | 11.46±9.65 | 0.866 |
|         |                   | Spike Amp | 27.72±15.85 | 25.50±14.81 | 0.735 |
|         |                   | Freq   | 23.67±24.74 | 17.82±20.77 | 0.091 |

RMP, resting membrane potential (mV); Amp, amplitude (mV); Freq, frequency (/min). *p < 0.050.

Fig. 3. The amplitude, AUC, and frequency were significantly decreased after administration of eupatilin in tension recordings of ileal (A) and colonic (B) smooth muscle strips.

**Bowel segment tension recordings**

Contractile waves were detected in bowel segments using tension recordings at proximal, middle, and distal sites of CM and LM. The contractions of bowel segments were reduced after administration of eupatilin in both the ileum and the colon (Fig. 4). The amplitude was significantly decreased after administration of 1 μM of eupatilin in CM (8.71±0.71 vs 3.59±1.29, p=0.002) and LM of ileal segment (8.87±0.44 vs 4.07±2.17, p=0.002). The AUC was decreased at 1 μM of eupatilin in CM (70.75±9.45 vs 39.64±19.95, p=0.005) and LM of ileal segment (70.18±10.35 vs 35.37±13.57, p=0.002). The frequency was also decreased after administration of 1 μM of eupatilin in CM (1.92±0.11 vs 1.45±0.14, p=0.002) and LM of ileal segment (1.83±0.10 vs 1.40±0.07, p=0.002). The amplitude, AUC and frequency were decreased significantly after administration of 2.5 and 5 μM of eupatilin in CM and LM of ileal segment (p < 0.050). Eupatilin reduced the amplitude of CM (17.37±2.40 vs 9.91±6.24, p=0.002) and LM of colonic segment (18.95±3.13 vs 9.83±7.27, p=0.002) at 1 μM. The AUC was also de-
Table 2. Tension recordings of muscle strips before and after administration of eupatilin

|       | CM                     | LM                      | p-value          |
|-------|------------------------|-------------------------|-----------------|
|       | Before                 | After                   | Before          | After                     |
| Ileum | 1 μM (n=5)             | 2.26±1.05               | 2.46±2.42       | 0.043*0.020*              |
|       | Amp 4.15±1.00          | 4.66±2.42               | 32.46±10.20     | 0.043*0.006*              |
|       | AUC 20.79±3.31         | 2.03±1.06               | 1.66±2.80       | 0.043*0.042*              |
|       | Freq 3.45±1.23         | 2.50±1.96               | 3.31±1.28       | 0.043*0.032*              |
| 2.5 μM| Amp 3.77±1.40          | 2.48±1.62               | 35.16±11.01     | 0.043*0.004*              |
|       | AUC 16.84±4.79         | 2.55±1.66               | 3.19±1.82       | 0.043*0.006*              |
|       | Freq 3.89±1.55         | 2.52±1.48               | 3.52±1.47       | 0.043*0.005*              |
| 5 μM  | Amp 4.20±0.94          | 0.40±0.61               | 6.70±2.49       | 0.043*0.003*              |
|       | AUC 19.90±4.11         | 1.11±1.85               | 33.23±9.47      | 0.043*0.004*              |
|       | Freq 4.16±1.34         | 0.84±1.48               | 3.52±1.47       | 0.043*0.005*              |
| Colon | 1 μM (n=14)            | 5.44±3.28               | 6.56±4.41       | 0.001*0.001*              |
|       | Amp 17.35±3.97         | 6.26±3.04               | 257.30±191.57   | 0.001*0.001*              |
|       | AUC 496.37±60.63       | 238.13±140.12           | 619.23±183.64   | 0.001*0.001*              |
|       | Freq 1.38±0.08         | 1.22±0.08               | 686.42±164.32   | 0.001*0.001*              |
| 2.5 μM| Amp 16.36±2.73         | 0.86±0.71               | 1.47±2.25       | 0.001*0.001*              |
|       | AUC 492.42±64.39       | 70.02±46.92             | 619.23±183.64   | 0.001*0.001*              |
|       | Freq 1.35±0.06         | 0.92±0.41               | 619.23±183.64   | 0.001*0.001*              |
| 5 μM  | Amp 20.55±3.92         | 0.14±0.19               | 0.92±0.41       | 0.001*0.001*              |
|       | AUC 500.34±72.31       | 8.95±12.48              | 6.26±8.89       | 0.043*0.043*              |
|       | Freq 1.43±0.08         | 0.43±0.59               | 6.26±8.89       | 0.043*0.043*              |

Table 2. Tension recordings of muscle strips before and after administration of eupatilin

Amp, amplitude (mN); AUC, area under the curve (sec×mN /min); Freq, frequency (/min). *p < 0.050.

Fig. 4. The amplitude, AUC, and frequency were significantly decreased after administration of eupatilin in tension recordings of ileal (A) and colonic (B) segments.

increased at 1 μM of eupatilin in CM (518.28±92.93 vs 397.06±178.87, p=0.004) and LM of colonic segment (620.45±134.37 vs 414.55±218.73, p=0.005). The frequency was decreased at 1 μM of eupatilin in CM (0.42±0.08 vs 0.25±0.10, p=0.001) and LM of colonic segment (0.43±0.09 vs 0.26±0.10, p=0.001). The amplitude, AUC and frequency were also decreased significantly after administration of 2.5 and 5 μM of eupatilin in CM and LM of colonic segment (p<0.050). Detailed analyses of bowel segment tension recordings are also described in Table 3. Eupatilin induced a dose-dependent decrease of the amplitude, AUC and frequency at ileal and colonic segment (Fig. 5).

Propagation Patterns

In ileal segment, antegrade propagation was observed 4.00±1.00, and retrograde propagation was 3.20±0.45 times per 10 waves. Mixed patterns were identified 2.80±1.30 times per 10 waves. After administration of eupatilin, antegrade propagation was 3.40±1.14, and retrograde propagation was observed 4.00±1.22 times per 10 waves. Mixed patterns were 2.60±1.52 per 10 waves, and there were no
Table 3. Tension recordings of bowel segments before and after administration of eupatilin

|       | CM          |        | LM          |        |
|-------|-------------|--------|-------------|--------|
|       | Before      | After  | Before      | After  |
| Ileum |             |        |             |        |
| 1 μM  | Amp         | 8.71±0.71 | 3.50±1.29   | 8.87±0.44 | 4.07±2.17 | 0.002*/0.002* |
|       | AUC         | 70.75±9.45 | 39.64±19.95 | 70.18±10.35 | 35.37±13.57 | 0.005*/0.002* |
|       | Freq        | 1.82±0.11 | 1.45±0.14   | 1.83±0.10 | 1.40±0.07 | 0.002*/0.002* |
| 2.5 μM| Amp         | 8.63±0.70 | 0.59±0.27   | 8.94±0.48 | 1.13±0.95 | 0.028*/0.028* |
|       | AUC         | 71.12±9.05 | 8.82±4.70   | 70.05±12.44 | 9.87±2.62 | 0.028*/0.028* |
|       | Freq        | 1.80±0.12 | 1.14±0.04   | 1.81±0.10 | 1.19±0.06 | 0.028*/0.028* |
| 5 μM  | Amp         | 8.79±0.77 | 0.08±0.13   | 8.80±0.44 | 1.00±0.13 | 0.028*/0.028* |
|       | AUC         | 70.37±10.69 | 0.53±0.88  | 70.32±8.98 | 1.14±1.36 | 0.028*/0.028* |
|       | Freq        | 1.84±0.10 | 0.47±0.76   | 1.84±0.11 | 0.56±0.61 | 0.046*/0.028* |
| Colon |             |        |             |        |
| 1 μM  | Amp         | 17.37±2.40 | 9.91±6.24   | 18.95±3.13 | 9.83±7.27 | 0.002*/0.002* |
|       | AUC         | 518.28±92.93 | 397.06±178.87 | 620.45±134.37 | 414.55±218.73 | 0.004*/0.005* |
|       | Freq        | 0.42±0.08 | 0.25±0.10   | 0.43±0.09 | 0.20±0.10 | 0.000*/0.000* |
| 2.5 μM| Amp         | 16.41±2.30 | 5.02±6.37   | 17.99±2.92 | 4.79±6.58 | 0.013*/0.013* |
|       | AUC         | 489.30±67.01 | 170.23±191.00 | 643.13±144.84 | 165.65±207.68 | 0.013*/0.013* |
|       | Freq        | 0.38±0.04 | 0.12±0.10   | 0.39±0.05 | 0.12±0.10 | 0.005*/0.007* |
| 5 μM  | Amp         | 18.96±1.71 | 1.05±1.30   | 20.56±2.99 | 1.26±1.62 | 0.028*/0.027* |
|       | AUC         | 566.58±115.54 | 8.08±12.64 | 582.66±116.89 | 8.77±14.14 | 0.028*/0.028* |
|       | Freq        | 0.50±0.06 | 0.07±0.15   | 0.50±0.11 | 0.10±0.14 | 0.027*/0.028* |

Amp, amplitude (mN); AUC, area under the curve (sec×mN /min); Freq, frequency (/min). *p < 0.050.

DISCUSSION

Here, we report that eupatilin (Stillen®) decreases human lower gastrointestinal motility. The RMP was hyperpolarized after administration of eupatilin in intracellular electrophysiologic recordings of ileum and colon samples. Eupatilin inhibited excitability by stabilizing the membrane potential and affected contractions of gastrointestinal smooth muscle. Tension recordings of smooth muscle strips and ileal or colonic segments also showed decreased contractility. Eupatilin has long been used to treat nonspecific gastric symptoms such as abdominal pain or diarrhea in oriental medicine. It has been reported to be protective against gastritis or peptic ulcers in experimental or clinical studies, and has been widely used as a tablet drug, Stillen®. The mucoprotective effects of eupatilin are mediated by increased mucus and prostaglandin (PG) secretion from the gastric mucosa and a the cytoprotective action is mediated by nitric oxide (NO) [17]. Eupatilin also has been reported to reduce the activation of human neutrophils induced by Helicobacter pylori [18] and suppress tumor proliferation by inducing cell cycle arrest [19-21]. However, the effects of eupatilin on gastrointestinal motility have not been previously studied, although there was a study differences of propagation patterns after administration of eupatilin (n=5, p > 0.050). In colonic segment, antegrade propagation was identified 6.00±0.71, and retrograde propagation was 1.40±0.55 times per 10 waves. Mixed patterns were observed 2.60±0.55 times per 10 waves. After administration of eupatilin, antegrade propagation was 5.20±0.84, and retrograde propagation was observed 1.60±0.55 times per 10 waves. Mixed patterns were 3.20±0.45 per 10 waves, and there were no differences of propagation patterns after administration of eupatilin (n=5, p > 0.050).

Fig. 5. Eupatilin induced a dose-dependent decrease in segmental tension recordings. The amplitude, AUC, and frequency of ileum (A) and colon (B) muscle contractions decreased as eupatilin increased from 0 to 1, 2.5 and 5 μM (■ circular muscle, □ longitudinal muscle).
showing non-toxic effects on the contractions of guinea pig ileum [14].

PGs are autocrine or paracrine lipid mediators produced by cyclooxygenase or lipoxygenase in fatty acid metabolism, and have various actions on smooth muscle. PGF₂α and PGE₁ increase gastrointestinal smooth muscle contractions by increasing the release of Ach from cholinergic nerve terminals. PGE₂ inhibits smooth muscle contractions through specific receptors, such as EP₁ or EP₂ [22,23]. Eupatilin is known to increase the release of PGE₂ [13], which might be one of the reasons for the observed relaxation of gastrointestinal smooth muscle contractions. However, some studies reported that PGE₂ depolarized membrane potential and enhanced excitability by increasing acetylcholine release [24]. NO is also involved in the cytoprotective effects of eupatilin on gastric mucous cells [15]. NO contributes to the maintenance of mucosal integrity, which might be mediated by vasodilatation and increased blood flow, or by inhibition of neutrophil-platelet interactions that lead to the formation of thrombosis and mucosal hemorrhages. Excessive NO could be cytotoxic, leading to uncontrolled vasodilatation, hypotension, damage to microvascular integrity of mucosa and increased free radicals [25]. NO has restrictive effects on gastrointestinal smooth muscle contractions by reducing noncholinergic and nonadrenergic neurotransmitter release and altering calcium concentrations in the ICC and smooth muscle cells [26]. PG or NO released from the mucosa might be responsible to decrease gastrointestinal motility in this study. But the reduction of contractions was observed in tension recordings of smooth muscle strips, in which mucosa and submucosa were removed. Another mechanism for direct action to smooth muscle cells can be involved in inhibition of contractions. Further studies are necessary to identify the relationships between of Stillen® or eupatilin, neuropeptides, and their effects on gastrointestinal motility.

IBS is the most common gastrointestinal disorder, characterized by abdominal discomfort with altered bowel function and increasing or decreasing intestinal motility. IBS is classified with 4 subtypes by predominant stool pattern, whether it is constipation or diarrhea [9]. It has been reported that IBS with constipation was associated with prolonged gut transit times and IBS with diarrhea was also related to intestinal dysmotility [27]. Although many therapies, such as increased dietary fiber, antidiarrheals, anti-spasmodics, probiotics, or antidepressants, have been used to relieve IBS symptoms, there have not been strong evidence of long-term efficacy [28,29]. As altered levels of 5-Hydroxytryptamine (HT) are related to bowel motility disorders, some effective drugs that act on 5-HT receptors have been introduced, and alosetron is a 5-HT₁ receptor antagonist that can improve the symptoms of IBS with diarrhea [30,31]. It inhibited MMC in murine small and large bowel in an in vitro study [11]. Although many 5-HT receptor related drugs have been withdrawn due to their side effects, alosetron has current Food and Drug Administration (FDA) approval. We found that Stillen® has inhibitory effects on human lower gastrointestinal motility, and it may improve symptoms related to increased bowel motility, and could also be used to treat IBS with diarrhea.

This study has some limitations. First, we used normal human bowel tissue, but experiments on bowels with IBS might allow us to better to verify the effects of Stillen® in disease. However, it is not easy to obtain IBS tissues because IBS is not treated by surgery. Second, the effects of Stillen® are not exactly those of eupatilin, so further experiments using eupatilin are necessary to explain a detailed mechanism of action. Third, as this was an in vitro study, further clinical trials for in patients should be performed to confirm the effects of the drug in IBS.

In conclusion, eupatilin (Stillen®) showed inhibitory effects on the human ileum and colon measured by intracellular recordings and smooth muscle and bowel segment tension recordings. We suggest that eupatilin (Stillen®) could be used for treating diseases with increased bowel motility, but further studies are necessary.

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