ARTICLE

YH12852, a Potent and Selective Receptor Agonist of 5-hydroxytryptamine, Increased Gastrointestinal Motility in Healthy Volunteers and Patients With Functional Constipation

Hyun A. Lee[1,2], Seol Ju Moon[3], Hyounggyoon Yoo[1], Mi Kyung Kim[4], Seong Bok Jang[4], Seoungoh Lee[4], Sohee Kim[4] and Howard Lee[1,2,5,*]

Gastrointestinal (GI) motility disorders are common, decreases quality of life, and imposes a substantial economic burden. YH12852 is a novel agonist of 5-hydroxytryptamine for the treatment of GI motility disorders. This phase I/IIa study assessed the tolerability, pharmacodynamic (PD) and pharmacokinetic (PK) profiles of YH12852. In the multiple dose (MD) cohort, healthy subjects and patients with functional constipation were randomized and received orally YH12852 at 0.3, 0.5, 1, 2, or 3 mg or prucalopride 2 mg or their matching placebo, once daily for 14 days after breakfast. In the multiple low-dose cohort (MLD), healthy subjects randomly received once-daily oral doses of YH12852 at 0.05 or 0.1 mg for 14 days after breakfast. Questionnaires, gastric emptying breath test for PDs, and plasma samples for PKs were collected. In the MD cohort, a total of 56 subjects (29 healthy volunteers and 27 patients with functional constipation) were randomized, of whom 48 completed the study. In the MLD cohort, a total of 16 healthy subjects were randomized, and 15 subjects completed the study. YH12852 increased the average weekly frequency of spontaneous bowel movements and loosened the stool. In addition, YH12852 increased quality of life satisfaction, and decreased severity of constipation symptom and GI symptoms. YH12852 was safe and well-tolerated up to 3 mg and showed nearly dose proportional PKs. In conclusion, YH12852 was safe and enhanced GI motility. YH12852 can be developed as an effective treatment option for GI motility disorders, including functional constipation. Further studies are warranted to confirm this possibility.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Five-hydroxytryptamine (5-HT) promotes gastrointestinal (GI) motility in the small and large intestines through the 5-HT receptor such as 5-HT$_4$ receptor. The 5-HT receptor is considered an attractive drug target to treat patients with constipation. YH12852 is a novel and highly selective agonist of the 5-HT$_4$ receptor.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This phase I/IIa study evaluated the tolerability, pharmacodynamic and pharmacokinetic profiles of YH12852 after multiple oral administration in healthy volunteers and patients with functional constipation.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ YH12852 was safe and well-tolerated. Multiple oral administration of YH12852 enhanced GI motility. Oral YH12852 was absorbed fast, and its exposure increased in a dose-proportional manner over 0.05–3 mg.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ YH12852 appeared to have a more potent prokinetic effect than other 5-HT$_4$ receptor agonists, such as velusetrag, tegaserod, cisaprid, and prucalopride. YH12852 can be developed as an effective treatment for functional constipation.

Chronic constipation, irritable bowel syndrome, and functional dyspepsia are collectively referred to as gastrointestinal (GI) motility disorders. GI motility disorders are a common and debilitating disease that profoundly decreases quality of

life and imposes a substantial economic burden.[1] Traditional medications, including bulk forming laxatives, osmotic laxatives, and stool softeners, have been used to treat GI motility disorders. Although laxatives are effective, some patients

[1]Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Hospital, Seoul, Korea; [2]Department of Transdisciplinary Studies, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea; [3]Center for Clinical Pharmacology, Biomedical Research Institute, Chonbuk National University Hospital, Jeonju, Korea; [4]Clinical Development Department, Research & Development Division, Yuhan Corporation, Seoul, Korea; [5]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea. *Correspondence: Howard Lee (howardlee@snu.ac.kr)

could experience adverse drug reactions.[2] Constipation is one of the most common GI motility disorders in Western countries.[3] The prevalence of constipation is higher in adults > 50 years of age than in younger adults of 18–35 years, and severe constipation is common in elderly women.[4,5] Current treatment strategies for constipation primarily aim to restore the normal neurogenic regulation of gut function. Five-hydroxytryptamine (5-HT) or serotonin, an important signaling molecule in the brain-gut axis, plays an important role in promoting GI motility, such as peristalsis, in the small and large intestines.[6–8] Although endogenous 5-HT is not essentially required for peristalsis or colonic migrating motor complexes, exogenous 5-HT agonists potently increase GI motility.[9–11] Five-HT signaling is mediated through the 5-HT receptor, of which 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, and 5-HT_7 subtypes are known to affect GI motility.[12,13] Particularly, the 5-HT_4 receptor subtype has been intensively studied in association with GI motility because it is distributed along the gut and plays a role in mediating peristalsis and mucosal secretion. Thus, the 5-HT_4 receptor is an attractive drug target to treat constipation.[14] To support this notion, 5-HT_4 receptor agonists, such as cisapride, tegaserod, and prucalopride, have been demonstrated to stimulate both whole gut transit and colonic transit, and were approved to treat patients with gastro-esophageal reflux disease, dyspepsia, or constipation.[15–17]

However, several 5-HT_4 receptor agonists were withdrawn from the market due to cardiovascular concerns. For example, cisapride was withdrawn from the global market in 2000 after multiple reports of life-threatening arrhythmias associated with prolonged corrected QT syndrome.[18] Likewise, tegaserod was withdrawn in 2007, although its relation with cardiovascular events remains inconclusive.[19] The limited selectivity of cisapride and tegaserod for the 5-HT_4 receptor could have contributed to the cardiovascular adverse effects commonly seen in patients treated with them.[5]

YH12852 is a novel, highly selective agonist of the 5-HT_4 receptor. YH12852 has 84 times higher binding affinity for the 5-HT_4 receptor than tegaserod. In the preclinical studies, YH12852 significantly improved motility in both upper and lower GI tracts, reduced visceral hypersensitivity, and showed an excellent safety profile.[20] YH12852 was also approximately three times more potent than prucalopride for contractile activity in the distal colon of guinea pigs (mean half maximal effective concentration: 4.2 vs. 13 nM).[20,21] Furthermore, all electrocardiogram (ECG) outputs were qualitatively and quantitatively normal in cynomolgus monkeys, which received a single oral dose of YH12852 up to at 60 mg/kg/day. YH12852 exhibited high selectivity for the human 5-HT_4 receptors, whereas YH12852 had no significant affinity for other 5-HT (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, or 5-HT_3) and non 5-HT receptors or channels. Consistent with the very low affinity of YH12852 with the hERG channel, it had no meaningful effect on blood pressure, heart rate, or ECG, as measured in male cynomolgus monkeys using a telemetry system after single oral administration of YH12852 up to 60 mg/kg. Furthermore, no significant QT prolongation was observed in a previous human study (ClinicalTrials.gov Identifier: NCT01870674). These findings suggest that the clinical use of YH12852 would be associated with a low cardiac safety risk.

The objective of this study was to evaluate the tolerability, pharmacodynamic (PD) and pharmacokinetic (PK) profiles of YH12852 after multiple oral administration in humans. To this end, we performed a phase I/IIa study in healthy volunteers and patients with functional constipation after multiple oral administrations of YH12852.

METHODS
Study subjects
Healthy volunteers and patients with functional constipation aged between 19 and 60 years (both inclusive) with a body mass index (BMI) of 18–5 kg/m^2 were screened. To be eligible for this study, healthy volunteers had to document ≤ 3 spontaneous bowel movements per week for at least 3 months, whereas patients with functional constipation should meet all of the modified Rome III functional constipation criteria for at least 3 months before the screening visit with a symptom onset at least 6 months before the diagnosis[22]: (1) lumpy or hard stools in at least 25% defecations, (2) < 3 defecations per week, and (3) loose stools rarely occurring without using laxatives. Subjects were excluded from the study if they had any of the following: presence or history of diseases or conditions that were associated with constipation, structural or postsurgical GI disorders, diseases or conditions that could affect GI motility or defecation, or medical history of cancer (other than basal cell or squamous cell carcinoma of the skin).

Study design
We performed a randomized, double-blind, placebo/active-controlled, parallel study in the multiple dose (MD) cohort and a randomized, double-blind, parallel study in the multiple low-dose (MLD) cohort (**Figure 1**). Healthy subjects and patients with functional constipation in the MD cohort were recruited in a 1:1 ratio by dose group, and each dose group received YH12852 at 0.3, 0.5, 1, 2, or 3 mg or prucalopride at 2 mg[23,24] or their matching placebo, all orally and once-daily, in a ratio of 1:1:1 for 14 days after breakfast. On the other hand, healthy subjects were recruited in the MLD cohort, and they randomly received once-daily oral doses of YH12852 at 0.05 or 0.1 mg in a ratio of 1:1 for 14 days after breakfast. The selection of the YH12852 doses was based on the results from the preclinical studies that oral YH12852 at 0.01–5.3 mg and 0.05–7.1 mg in humans weighing 60 kg would improve lower and upper GI motility, respectively. Furthermore, in the previous phase I study (ClinicalTrials.gov identifier: NCT01870674), repeated administration of YH12852 was safe and well-tolerated up to 3 mg, which supported the parallel design over the ascending dose design in the present phase I/IIa study.

Eligible subjects were admitted to the Clinical Trials Center at Seoul National University Hospital 1 day before study drug administration and discharged after all of the scheduled procedures were completed.

This study was conducted in full compliance with the principles stipulated in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practices guideline and other
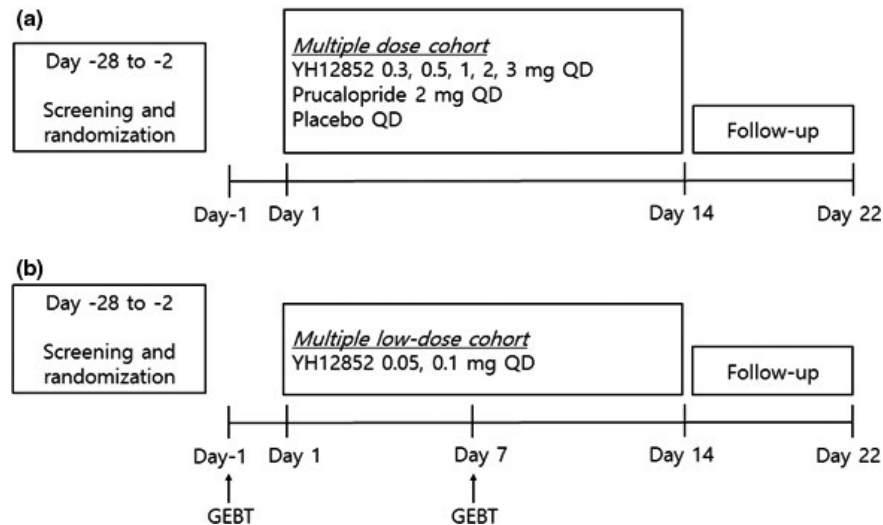
Figure 1 Study flow of multiple dose cohort (**a**) and multiple low-dose cohort (**b**) GEBT, ^{13}C-Spirulina platensis Gastric Emptying Breath Test.

regulatory requirements (clinicaltrials.gov registry numbers: NCT02538367). The protocol was approved by the Institutional Review Board at Seoul National University Hospital, and all study participants provided written informed consent before any study procedure was performed.

Tolerability assessments
Tolerability was evaluated throughout the entire study based on physical examination, vital signs, 12-lead ECG, chest X-ray, and laboratory tests (hematology, serum chemistry, coagulation, and urinalysis). Twelve-lead ECGs were obtained on day −2, day −1 (0, 1, 2, 3, 4, 5, 8, 10, 12, 14, and 24 hours post-placebo), day 10, day 14 (predose, 1, 2, 3, 4, 5, 8, 10, 12, 14, and 24 hours post-YH12852), and day 16 (before discharge). ECGs on days −1 and 14 were time-matched. We also performed a concentration-QT prolongation analysis by dose. QT interval was converted using the Fridericia correction formula.

Pharmacodynamic assessments
All of the subjects completed a daily bowel habit diary during the baseline (days −8 to −2) and the treatment periods, which documented the frequency of spontaneous bowel movements (SBMs), stool consistency based on the 7-point Bristol Stool Form Scale with 1 and 7 being the hardest and softest stools, respectively,[25] and feeling of incomplete evacuation and straining during bowel movement based on a 5-point Likert Scale (i.e., 0 = never or rarely, 1 = sometimes, 2 = often, 3 = most of the times, and 4 = always).[26] The scores on the Likert scale was averaged in each subject over the day and then averaged again over the days in the corresponding week. The outcomes of the questionnaires, such as the Patient Assessment of Constipation-Symptom (PAC-SYM), the Patient Assessment of Constipation-Quality of Life (PAC-QOL), and the Patient Assessment of Gastrointestinal Disorders Symptom Severity (PAGI-SYM), were also evaluated at the end of baseline and on day 14.[27] The proportion of responders, defined as those with an increase ≥ 1 from baseline in weekly frequency of SBMs, was evaluated.

In the MLD cohort, the prokinetic effect of YH12852 was explored. The speed and extent of gastric emptying (GE) at baseline and on day 7 was measured using the ^{13}C-Spirulina platensis gastric emptying breath test (GEBT; Cairn Diagnostics, Brentwood, TN).[26] After an overnight fast, all participants consumed the test meal containing ^{13}C-Spirulina, powdered egg, and saltine crackers. On day 7, YH12852 was administered right after the test meal. Exhaled air samples were collected before the test meal, and at 45, 90, 120, 150, 180, and 240 minutes after the test meal. Times for half and 10% GE (t_{50} and t_{10}, respectively), area under the percentage of GE-time curve (gAUC) and GE rate at sampling times were estimated. GE rate was the percent ^{13}C dose excreted per minute multiplied by 1,000 (percent ^{13}C dose excreted per minute multiplied by 1,000 (kPCD) min^{-1}).

Pharmacokinetic assessments
Approximately 7 mL of whole blood were collected using a tube containing sodium heparin anticoagulant and centrifuged immediately after collection at the study site (3,000 rpm, 2–4°C, 15 minutes) to obtain plasma, which was then stored at −70°C until analysis. In the MD cohort, sampling times on days 1 and 14 were 0 (i.e., predose), 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, and 24 hours postdose. On day 14, additional blood samples were obtained at 36, 48, and 72 hours postdose. Furthermore, predose blood samples were collected on days 5, 10, 12, and 13 to determine the trough plasma concentrations of YH12852. In the MLD cohort, sampling times were the same as in the MD cohort on days 1 and 14, whereas trough blood samples were collected only on days 5 and 13.

The plasma concentrations of YH12852 were determined using a validated analytical method at BioCore (Seoul, South Korea). YH12852 was extracted from human plasma using protein precipitation. Two hundred

µL of plasma was transferred into an Eppendorf micro centrifuge tube and 500 µL of acetonitrile was added to precipitate proteins. The suspension was properly mixed using a vortex mixer for 1 minute and centrifuged at 13,000 rpm for 5 minutes. Then, the organic phase was transferred to glass tube and completely evaporated to dryness under N_2 gas. The dry residue was reconstituted with 200 µL of 50% methanol, and the reconstituted solution was centrifuged at 13,000 rpm for 5 minutes. Five µL of clear supernatant was injected into a liquid chromatography tandem mass spectrometry system (LC: Prominence UFLC XR, Shimadzu, Japan; MS/MS: 5500 QTRAP, AB SCIEX, USA), which determined the concentration of YH12852. The calibration curve of YH12852 was linear in the range of 30–20,000 pg/mL. A mobile phase containing 10 mM ammonium acetate and acetonitrile at flow rates of 0.12 mL/min and 0.08 mL/min, respectively, and an analytical column (C_{18}, 2.1 mm $\times$ 50 mm^2, 3 µm). A positive ion multiple-reaction-monitoring mode was used to detect analyte (*m/z* 400.3 → 296.3) and internal standard (YH12852-d_5, *m/z* 405.3 → 301.3).

Pharmacokinetic analysis

The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly obtained from the observed data. The elimination rate constants (λ_z) were determined by linear regression of the terminal portion (using at least three points) of the log-transformed plasma concentration-time curves. Then, the terminal half-life was ln(2) divided by λ_z. The area under the curve (AUC) from time 0 to last measurable time (AUC_{last}) was calculated using the linear-up and log-down trapezoidal method. AUC extrapolated to infinity (AUC_{inf}) was obtained $AUC_{last} + C_{last}/\lambda_z$, where C_{last} was the last quantifiable plasma concentration. The apparent oral clearance was the dose divided by AUC_{inf}. The PK parameters were determined not only after a single administration (day 1) but also under steady-state condition (day 14). The accumulation ratio (R) was calculated for C_{max} and AUC as the ratio of C_{max} during a dosing interval at steady-state ($C_{max,ss}$; i.e., day 14) to C_{max} on day 1 and $AUC_{\tau,ss}$ (AUC on day 14) to AUC_{last} on day 1, respectively. The attainment of steady-state was assessed based on visual inspection of the trough concentrations on days 5, 10, 12, and 13 in the MD cohort, and days 5, 13, and 14 in the MLD cohort. The PK parameters were estimated using a noncompartmental analysis option provided in the Phoenix WinNonlin (version 6.4; Certara USA, Princeton, NJ).

Statistical analysis

Demographic characteristics, daily bowel habits at baseline, results from the questionnaires, and PK parameters were summarized using descriptive statistics. Changes from baseline in average weekly number of SBMs, stool consistency, and sensation of incomplete evacuation during bowel movements were compared between the treatment groups by week using an analysis of covariance model that included baseline as a covariate or the Student's *t*-test in the MD and MLD cohorts, respectively. In the MD cohort, changes from baseline in average weekly number of SBMs and stool consistency were also compared between healthy subjects and patients with constipation using an analysis of covariance model that included baseline as a covariate. Additionally, changes from baseline on day 7 in GE rate, t_{50}, t_{10}, and gAUC were summarized using descriptive statistics, and tested for statistical significance within each dose group in the MLD cohort. The PK linearity of YH12852 was assessed using a power model for log-transformed C_{max}, $C_{max,ss}$, AUC_{last}, and $AUC_{\tau,ss}$. PK linearity was declared when the slope was not different from 1, and its 90% confidence interval entirely contained 1. *P* values < 0.05 were considered statistically significant. All the statistical analyses were performed using the SAS software (version 9.2; SAS Institute, Cary, NC).

RESULTS

Study subjects

In the MD cohort, a total of 56 subjects (16 men and 40 women; 29 healthy volunteers; and 27 patients with functional constipation) were randomized, 48 (85.7%) subjects completed the study as defined in the study protocol, and 8 subjects were dropped because of withdrawal of informed consent (*n* = 3), inability to comply with the study protocol (*n* = 1), or other reasons (*n* = 4). The mean (SD) age, height, body weight, and BMI of the 56 subjects were 28.0 (7.4) years, 165.1 (8.2) cm, 59.7 (9.2) kg, and 21.8 (1.9) kg/m^2, respectively. In the MLD cohort, a total of 16 healthy subjects (3 men and 13 women) were randomized, 15 (93.8%) subjects completed the study as defined in the study protocol, and 1 subject was dropped due to withdrawal of informed consent. None of the dropouts in both the MD and MLD cohorts were because of tolerability or safety issues. The mean (SD) of age, height, body weight, and BMI in those 16 subjects were 24.6 (3.8) years, 163.4 (7.7) cm, 57.7 (8.3) kg, and 21.5 (2.0) kg/m^2, respectively.

Tolerability assessments

YH12852 was safe and well-tolerated in all subjects. The proportion of subjects who reported treatment-emergent adverse events (AEs was comparable between placebo and YH12852 up to 2.0 mg, except for abdominal pain, which occurred more frequently in the YH12852 groups (**Table 1**). The most frequent treatment-emergent AEs after YH12852 were diarrhea, headache, nausea, vomiting, and dizziness. No serious AEs occurred in any treatment group. Likewise, no clinically significant changes were noted in vital signs, ECGs, physical examinations, or clinical laboratory tests. Furthermore, no discernible concentration-Fridericia correction formula prolongation relationship was found in all of the YH12852 doses (**Figure S1**).

Pharmacodynamic assessments

Multiple doses of YH12852 greatly increased the average weekly frequency of SBMs from baseline, and the increase in SBMs in the first week by YH12852 at 1.0–3.0 mg was significantly greater than that by placebo (**Figure 2A**). Likewise, the stool became softer after YH12852 and prucalopride treatments, and this change was significantly more marked than in the placebo group (**Figure 2B**). Changes

Table 1 Treatment-emergent adverse events by treatment group

Cohort		Multiple dose							Multiple low-dose	
		YH12852						Prucalopride	YH12852	
Adverse events	Placebo (n = 7)	0.3 mg (n = 8)	0.5 mg (n = 7)	1.0 mg (n = 8)	2.0 mg (n = 8)	3.0 mg (n = 8)	2 mg (n = 8)	0.05mg (n = 8)	0.1 mg (n = 8)
Any	5 (71.4)	7 (87.5)	6 (85.7)	7 (87.5)	6 (75.0)	8 (100)	8 (100)	5 (62.5)	5 (62.5)
Gastrointestinal disorders									
Nausea	1 (14.3)	4 (50.0)	2 (28.6)	2 (25.0)	3 (37.5)	1 (12.5)	3 (37.5)	1 (12.5)	0 (0.0)
Diarrhea	1 (14.3)	2 (25.0)	2 (28.6)	3 (37.5)	2 (25.0)	3 (37.5)	1 (12.5)	0 (0.0)	0 (0.0)
Abdominal pain	0 (0.0)	1 (12.5)	3 (42.9)	2 (25.0)	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)	0 (0.0)
Abdominal discomfort	1 (14.3)	2 (25.0)	2 (28.6)	0 (0.0)	0 (0.0)	3 (37.5)	3 (37.5)	0 (0.0)	1 (12.5)
Vomiting	0 (0.0)	1 (12.5)	2 (28.6)	2 (25.0)	1 (12.5)	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)
Dyspepsia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nervous system disorders									
Headache	2 (28.6)	5 (62.5)	5 (71.4)	4 (50.0)	6 (75.0)	4 (50.0)	6 (75.0)	1 (12.5)	3 (37.5)
Dizziness	1 (14.3)	3 (37.5)	2 (28.6)	3 (37.5)	3 (37.5)	1 (12.5)	3 (37.5)	1 (12.5)	0 (0.0)
Musculoskeletal and connective tissue disorders									
Myalgia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	1 (12.5)	0 (0.0)
Respiratory disorders									
Nasal congestion	0 (0.0)	1 (12.5)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Severity*									
Mild	15	36	32	34	29	31	28	10	12
Moderate	1	1	4	1	1	0	1	0	0
Severe	0	0	0	0	0	0	0	0	0

The data are presented as the number of subjects (%).
*Severity is displayed as number of events.

from baseline on day 7 in average weekly frequency of SBMs and stool consistency were comparable between healthy subjects and patients with constipation (**Table S2**). Furthermore, the increase in average weekly frequency of SBMs and softening of the stool by YH12852 was dose-dependent overall. Likewise, the sensation of incomplete evacuation during bowel movement was decreased after YH12852, particularly at 1.0–3.0 mg, and this decrease was numerically greater than in the placebo group, although it failed to reach statistical significance (**Figure 2C**). As a result, the proportion of responders, defined as those with an increase ≥ 1 from baseline in weekly frequency of SBMs, was 100% for the 0.5–2.0 mg dose groups throughout the study duration (i.e., 2 weeks) whereas it was only 40% in the placebo group (**Figure 3**). Similarly, the severity of constipation symptoms (PAC-SYM) was more decreased in subjects treated with YH12852 than in the placebo group, and the quality of life satisfaction (PAC-QOL) was more improved in subjects treated with YH12852 than in the placebo group during the 2-week treatment period, although none of them was statistically significant (**Table S3**). However, the improvements in those PD assessments appeared to lessen during week 2, which also became statistically insignificant. On the other hand, the GI disorders symptoms (PAGI-SYM) was not more decreased in subjects treated with YH12852 than in the placebo group.

In the MLD cohort, YH12852 accelerated GE at all doses, particularly after 0.1 mg. For example, administration of YH12852 at 0.1 mg for 7 days significantly reduced t_{10} and t_{50} (i.e., times required for 10 and 50% GE), by 15.2 and 21.4 minutes from baseline, respectively (**Table 2**). Accelerated GE by YH12852 was also clearly supported by the increase in kPCD from baseline, particularly after 0.1 mg at 45–120 minutes after meal (7.0–9.8 kPCD/min, **Table 2**).

Pharmacokinetic assessments
YH12852 was rapidly absorbed after oral administration, and its C_{max} was reached in 2.9–4.0 hours postdose (**Figure 4** and **Table 3**). Steady-state was attained on day 5 after multiple oral administrations (**Figure 4**). For example, the average trough concentrations on day 5 for 0.3, 0.5, 1, 2, and 3 mg in the MD cohort were 67.2, 219.0, 252.0, 415.8, and 839.6 pg/mL, respectively, which were similar to the average trough concentrations on day 14 (73.1, 205.6, 236.5, 358.8, and 803.3 pg/mL, respectively, for 0.3, 0.5, 1, 2, and 3 mg). The systemic exposure to YH12852 after multiple oral administrations for 14 days was increased in a dose-dependent manner over 0.05–3 mg (i.e., the 90% confidence intervals) of the slope of the log-transformed C_{max}, $C_{max,ss}$, and AUC_{last} included 1.0 (C_{max}: 0.9078–1.0645, $C_{max,ss}$: 0.8669–1.0477, and AUC_{last}: 0.9312–1.1362), but $AUC_{\tau,ss}$ was increased in a less than dose proportional manner (the 90% confidence intervals: 0.7521–0.9043). When given once daily, YH12852 was not accumulated markedly in the body, particularly in the MD cohort (i.e., accumulation ratio: 1.0–1.3 for C_{max}; 1.2–1.6 for AUC).

Multiple Dose Cohort
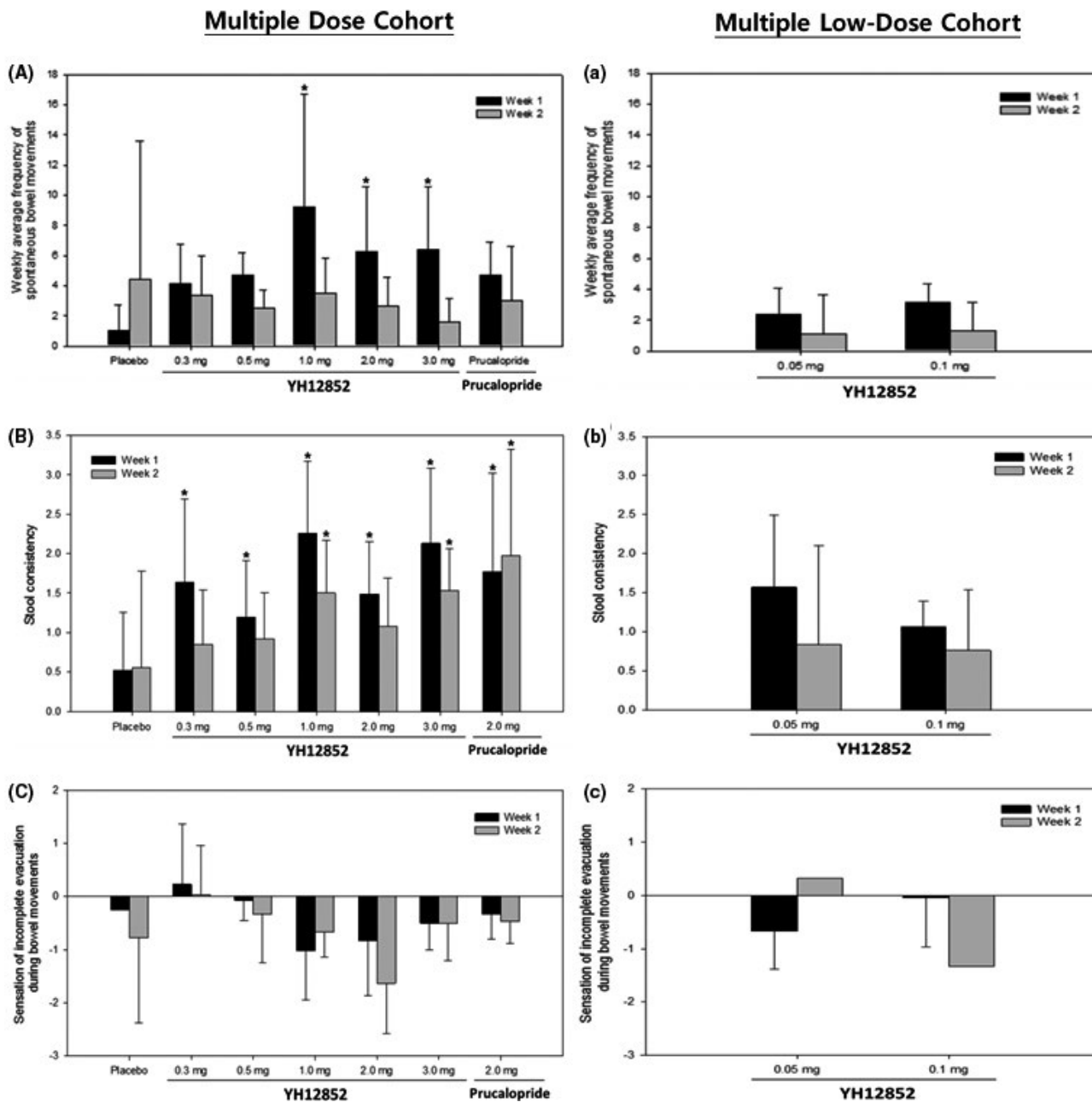
Multiple Low-Dose Cohort

Figure 2 Changes from baseline in weekly average frequency of spontaneous bowel movements, stool consistency based on the Bristol Stool Form Scale and sensation of incomplete evacuation during bowel movement after treatment ((**A**), (**B**), (**C**): multiple dose cohort; (**a**), (**b**), (**c**): multiple low-dose cohort). The error bars represent the SDs. *Pvalue < 0.05 vs. placebo.

DISCUSSION

This phase I/IIa study showed that multiple oral doses of YH12852 are safe, well-tolerated up to 3 mg, increases bowel movements, and softens the stool with nearly dose proportional PKs in healthy subjects and patients with functional constipation. All AEs resolved spontaneously without any treatment, and no serious AEs occurred during the entire study period (**Table 1**). The most frequent AEs, such as headache and GI events, in our study were commonly observed in previous studies with other 5-HT$_4$ receptor

agonists in healthy volunteers and patients with chronic constipation.[28–32] Furthermore, no clinically significant changes were noted after YH12852 administration in the clinical laboratory tests, physical examination, ECG findings, or vital signs.

YH12852 in the MD cohort markedly improved constipation signs and symptoms as measured by SBMs frequency, stool characteristics, and patient-reported satisfaction questionnaires. Evidence is that the change from baseline in the average weekly frequency of SBMs was significantly higher in the YH12852 group than in the placebo group, particularly
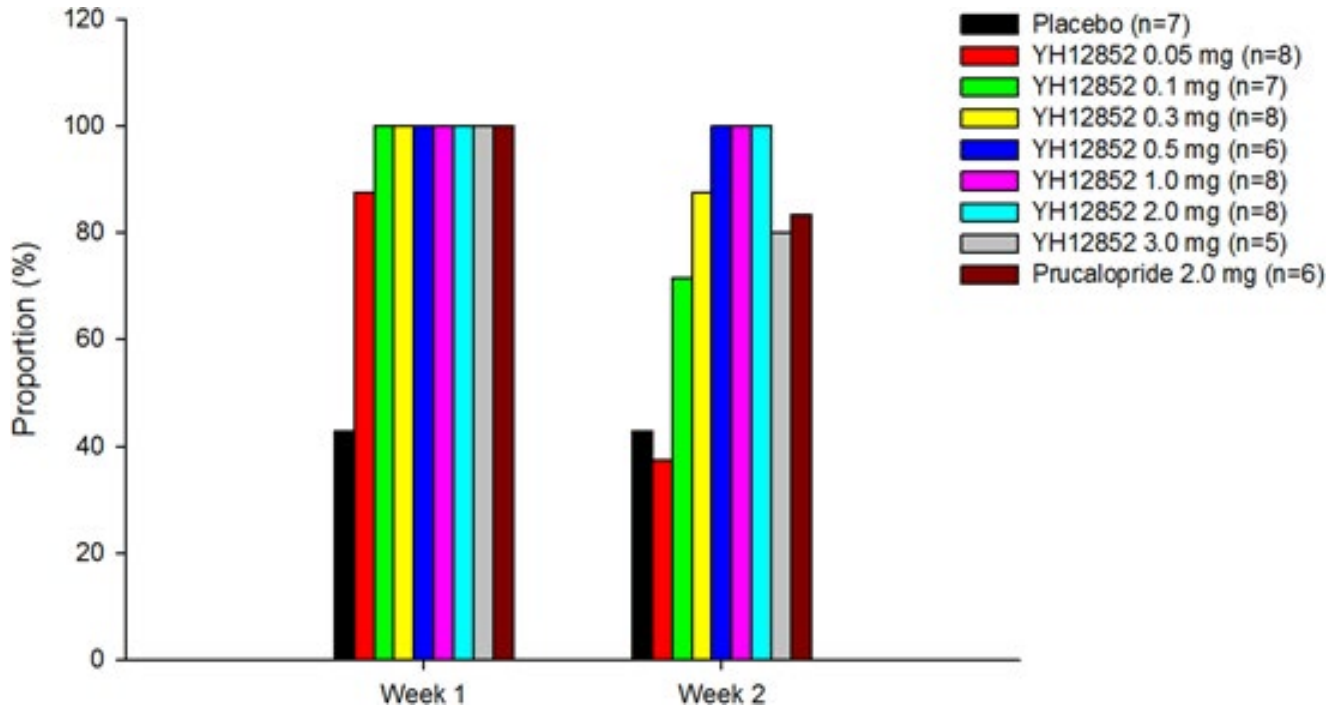
Figure 3 Proportion of responders for weekly average frequency of spontaneous bowel movements. Responders are defined as subjects with an increase ≥ 1 from baseline in weekly average frequency of spontaneous bowel movements.

Table 2 Changes from baseline on Day 7 in the parameters of the ^{13}C-Spirulina platensis gastric emptying breath test (multiple low-dose cohort)

Parameter	Time after meal, minutes	YH12852 0.05 mg (*n* = 8)	YH12852 0.1 mg (*n* = 7)
t_{10}, minutes	NA	−4.3 ± 18.8	−15.2 ± 15.9*
t_{50}, minutes	NA	−9.0 ± 27.2	−21.4 ± 20.8*
gAUC, %·minutes	NA	7.8 ± 35.4	21.0 ± 24.9*
GE rate, kPCD min^{-1}	45	2.3 ± 8.2	7.0 ± 8.4*
	90	2.5 ± 15.4	9.8 ± 12.0*
	120	2.9 ± 17.5	8.8 ± 12.2*
	150	3.7 ± 17.4	7.1 ± 11.4
	180	2.0 ± 13.2	0.1 ± 10.3
	240	−2.2 ± 8.0	−2.2 ± 12.6

The data are presented as arithmetic mean ± SD.
gAUC, the area under the percentage of gastric emptying-time curve; GE, gastric emptying; kPCD, percent ^{13}C dose excreted per minute multiplied by 1,000; NA, not applicable; t_{10}, time for 10% gastric emptying; t_{50}, time for half gastric emptying.
*Pvalue < 0.05 vs. baseline.

after the first week of treatment (**Figure 2a**). This prokinetic effect of YH12852 was coupled with statistically greater stool softening (**Figure 2b**) and numerically larger improvements in constipation and GI symptoms and quality of life (**Table S3**). Consequently, many of YH12852-treated subjects experienced an increase ≥ 1 from baseline in weekly frequency of SBMs, particularly after 1 week of treatment at 0.05–3 mg

(**Figure 3**). The improvements in PD assessments of YH1252 appeared to decrease during week 2. The decrease in efficacy after repeated administration, a phenomenon known as tachyphylaxis, has been reported with 5-HT$_4$ receptor agonist (e.g., tegaserod).[33] Tachyphylaxis may be caused by receptor desensitization due to 5-HT receptor induction. In case of YH12852, an increase in the availability of 5-HT might induce receptor desensitization, reducing the efficacy of YH12852 at week 2.

Prucalopride, an active comparator in this study, also increased the average weekly frequency of SBMs from baseline, but the increase was not significantly greater than that by placebo (**Figure 2a**). Similar increases in average weekly frequency of SBMs have been reported with other oral 5-HT$_4$ receptor agonists. For example, in a 4-week phase II study in patients with chronic idiopathic constipation, velusetrag 15, 30, and 50 mg increased average weekly frequency of SBMs more significantly than placebo.[34] Likewise, in a 4-week phase IIa study in patients with chronic idiopathic constipation, multiple twice-daily naronapride (ATI-7505) at 20, 40, 80, and 120 mg resulted in a greater average weekly frequency of SBMs than placebo during the first week.[35] Therefore, YH12852, which increased weekly SBMs at much lower doses of 1.0–3.0 mg, appeared to be more potent than prucalopride, velusetrag, and naronapride.

YH12852 shortened GE time even at very low doses of 0.05 and 0.1 mg (**Table 2**). Similar reductions in GE time have been reported with other 5-HT$_4$ receptor agonists. For example, multiple dosing of velusetrag at 15, 30, and 50 mg shortened GE time after 6 consecutive days in healthy subjects with an average reduction in t_{50} of
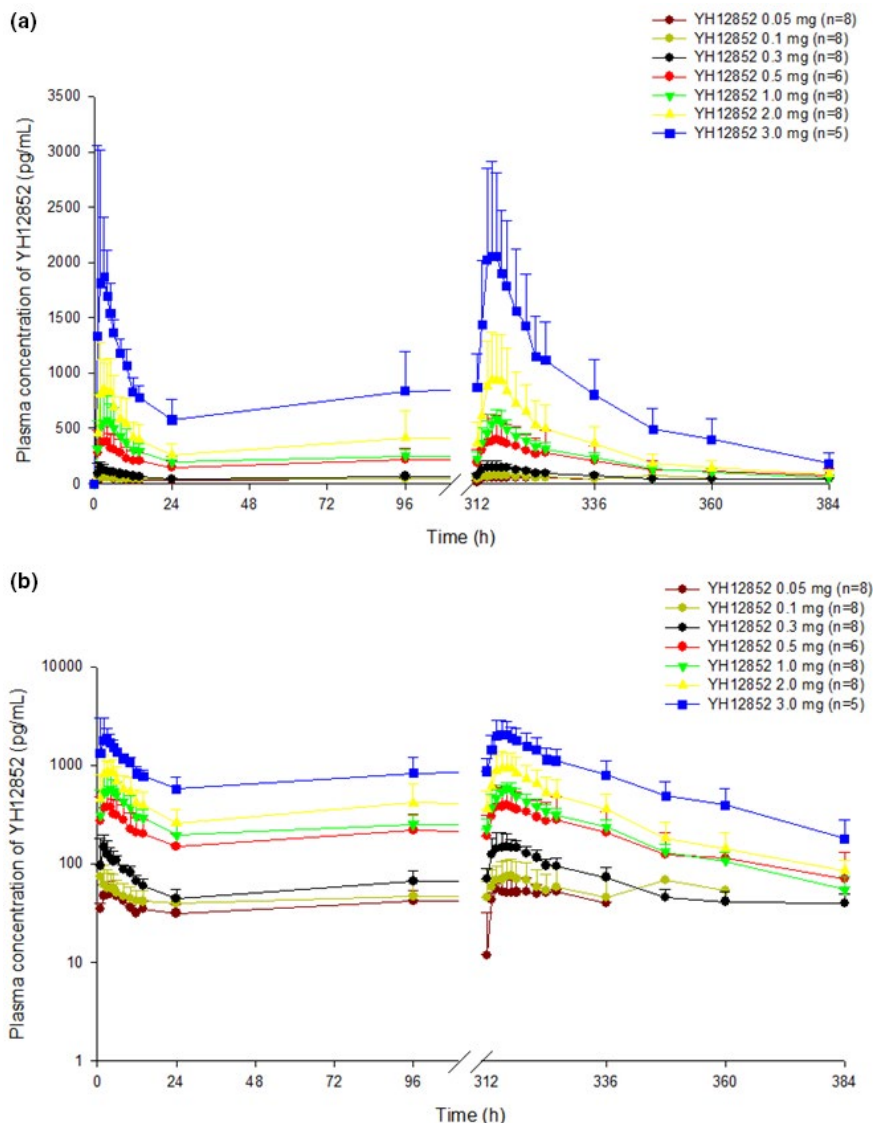
Figure 4 Mean plasma concentration-time profiles of YH12852 by dose ((**a**): linear scale, (**b**): semi-log scale). The error bars represent the SDs.

25 minutes (~ 19% acceleration), which was considered clinically beneficial.[36] Likewise, multiple oral dosing of tegaserod at 6 mg (a twice-daily dosing for 3 days and a daily dosing on the fourth day) in healthy male subjects significantly decreased GE time by 27% (*P* value < 0.001 vs. placebo).[37] However, cisapride, another 5-HT$_4$ agonist, was not effective in accelerating GE in healthy subjects.[38] Again, YH12852, which accelerated GE at such lower doses as 0.05 and 0.1 mg, appeared more potent than velusetrag, tegaserod, and cisapride.

Oral YH12852 was absorbed fast after administration and its exposure increased in a dose-proportional manner over 0.05–3 mg (**Figure 4**). YH12852 attained a steady-state after the fifth administration (**Figure 4**). Once-daily oral dosing of YH12852 did not result in a marked accumulation in the body, particularly in the MD cohort (i.e., accumulation ratio: 1.0–1.3 for C$_{max}$; 1.2–1.6 for AUC).

The present study had several limitations. First, we enrolled only a small number of subjects in each dose group due to the exploratory nature of the study. This may explain why we failed to see statistically significant differences between placebo and YH12852 in the sensation of incomplete evacuation during bowel movement and PAC-QOL, although YH12852 resulted in numerically greater improvements than placebo. Second, because the ^{13}C-Spirulina platensis GEBT was performed only in the MLD cohort, it is not clear if and how much higher doses of YH12852 could accelerate GE. However, changes from baseline in average weekly frequency of SBMs and stool consistency in the MD cohort were greater than those in the MLD cohort. Thus, although we did not perform the ^{13}C-Spirulina platensis GEBT in the MD cohort, the prokinetic effects of YH12852 on GE in the MD cohort are most likely to be greater than those in the MLD cohort. Third, we did not assess colonic transit, although

Table 3 Pharmacokinetic parameters of YH12852

Parameter	Day 1				Day 14							
	T_{max}, hours	C_{max}, pg/mL	AUC_{last}, pg·h/mL	$t_{1/2}$, hours	$T_{max,ss}$, hours	$C_{max,ss}$, pg/mL	AUC_τ, pg·h/mL	AUC_{inf}, pg·h/mL	$t_{1/2}$, hours	Cl_{ss}/F, L/h	R_{Cmax}	$R_{AUC\tau}$
Multiple dose cohort												
0.3 mg	3.8 ± 1.8	137.5 ± 39.1	1624.1 ± 434.3	20.8 ± 6.5	3.9 ± 2.0	170.7 ± 47.9	2,568.1 ± 582.2	5065.6 ± 1884.6	25.0 ± 13.2	123.2 ± 32.4	1.3 ± 0.5	1.6 ± 0.5
0.5 mg	2.9 ± 1.2	419.8 ± 167.0	5550.5 ± 2194.4	20.9 ± 7.6	3.7 ± 1.6	423.0 ± 211.6	6980.8 ± 4128.1	15081.7 ± 11198.8	28.4 ± 7.8	90.4 ± 42.3	1.1 ± 0.6	1.3 ± 0.8
1.0 mg	3.9 ± 1.1	657.4 ± 238.1	8108.2 ± 2578.1	21.3 ± 7.9	3.9 ± 1.0	608.6 ± 85.0	8732.3 ± 1155.4	16458.3 ± 3344.4	27.1 ± 7.0	116.5 ± 17.2	1.1 ± 0.5	1.2 ± 0.4
2.0 mg	3.0 ± 1.5	1037.0 ± 305.1	11454.6 ± 3900.0	20.8 ± 6.2	3.5 ± 1.2	996.7 ± 409.6	14244.6 ± 5937.2	24266.9 ± 10517.3	23.2 ± 6.0	162.9 ± 65.8	1.0 ± 0.4	1.3 ± 0.4
3.0 mg	3.2 ± 1.9	2372.5 ± 1219.4	23835.5 ± 4561.2	21.3 ± 7.7	3.6 ± 1.1	2188.4 ± 739.7	31454.8 ± 10818.2	57272.2 ± 21929.5	22.9 ± 4.2	103.9 ± 31.5	1.1 ± 0.7	1.4 ± 0.5
Multiple low-dose cohort												
0.05 mg	4.0 ± 0.0	59.1 ± 4.6	584.6 ± 38.8	13.0 ± 5.3	4.0 ± 1.4	58.8 ± 19.7	1014.0 ± 337.6	2675.7 ± 356.3	36.0 ± 9.2	54.5 ± 21.1	1.3 ± 0.1	2.2 ± 0.2
0.1 mg	4.0 ± 1.1	63.3 ± 27.6	591.6 ± 500.9	13.7 ± 10.2	4.0 ± 1.4	81.0 ± 36.9	1356.7 ± 708.9	2759.8 ± 1823.8	21.8 ± 8.8	85.5 ± 28.7	1.2 ± 0.3	3.0 ± 1.9

The data are presented as arithmetic mean ± SD.
AUC_{inf}, area under the plasma concentration–time curve from time 0 to infinity; AUC_{last}, area under the plasma concentration–time curve from time 0 to last measurable time; AUC_τ, area under the plasma concentration–time curve during a dosage interval (t); $C_{max,ss}$, peak plasma concentration at steady state; C_{max}, peak plasma concentration; $R_{AUC\tau}$, accumulation ratio of AUC_τ; R_{Cmax}, accumulation ratio of C_{max}; $t_{1/2}$, terminal half-life; $T_{max,ss}$, time to reach $C_{max,ss}$ after drug administration at steady state; T_{max}, time to reach C_{max} after drug administration.

GE was evaluated using GEBT as an exploratory measure of the general prokinetic effect of YH12852. However, colonic transit time is a clinically more meaningful measure than GE time. Further clinical studies are warranted to test if the prokinetic effect of YH12852 on GE is translated into shortened colonic transit time in patients with constipation.

In conclusion, oral YH12852 at 0.05–3 mg was safe and well-tolerated after multiple once-daily administration for 14 days in healthy volunteers and patients with functional constipation. Multiple administration of YH12852 enhanced GI motility, and YH12852 showed a potent prokinetic effect. Overall, YH12852 can be developed as an effective treatment for functional constipation. Further studies are warranted to confirm this possibility.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Conflict of Interest. M.K., S.J., S.L., and S.K. are employees of Yuhan Co. All other authors declared no competing interests for this work.

Author Contributions. H.A.L. wrote the manuscript. M.K., S.J., S.L., S.M, and H.L. designed the research. S.M., H.Y., and H.L. performed the research. H.A.L., H.Y., S.J., S.K., and H.L. analyzed data.

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