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ORIGINAL ARTICLE

Effect of single and multiple doses of elobixibat, an ileal bile acid transporter inhibitor, on chronic constipation: A randomized controlled trial

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Keywords gastroenterology, pharmacodynamics, pharmacokinetics, phase I, randomized controlled trial

AIMS

Elobixibat is a minimally absorbed ileal bile acid transporter inhibitor. This study aimed to investigate the safety, tolerability, efficacy, pharmacokinetics and pharmacodynamics of elobixibat in Japanese patients with chronic constipation.

METHODS

This study consisted of single-dose and multiple-dose tests with a dose-escalating design. Sixty patients including females and males were randomized into five dose levels of elobixibat (2.5, 5, 10, 15 or 20 mg, $n = 10$ per level) and corresponding placebo ($n = 2$ per group). A crossover design was used to examine food effect in single-dose test. Patients received test tablets once daily for 14 days in multiple-dose test. We assessed pharmacokinetic-dose proportionality, levels of serum high- and low-density lipoprotein cholesterol and plasma 7α-hydroxy-4-cholesten-3-one (C4), food effect and sex-specific effect. Adverse events and bowel functions such as bowel movements, stool consistency and straining were also evaluated.

RESULTS

Food consumption reduced systemic exposure by around 80% [e.g. least squares mean (ratio of breakfast/no breakfast) maximum plasma concentration: 0.2085 (90% confidence interval, 0.1371–0.3172) at 15 mg] while increased plasma C4 level ($P < 0.001$). In the multiple-dose test, elobixibat reduced low-density lipoprotein cholesterol and increased C4 whilst unaltering high-density lipoprotein cholesterol level. The increased spontaneous bowel movement frequency was correlated with higher dosage and higher C4 level ($R^2 = 0.5929$ at Week 2). Adverse events were mainly gastrointestinal symptoms, most of which were mild.

CONCLUSIONS

Elobixibat should be taken before breakfast. Once-daily administration of elobixibat was found to be safe and tolerated up to 20 mg in female and male patients with chronic constipation.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Elobixibat is a locally acting inhibitor of ileal bile acid transport.
- The dual action of elobixibat – enhancing colonic secretion and motility – has garnered interest in the treatment of constipation.
- A few clinical studies, which mainly recruited female patients with chronic constipation, have shown the efficacy and safety of elobixibat.

WHAT THIS STUDY ADDS

- Elobixibat increased bile acid synthesis associated with the number of spontaneous bowel movements and improved other constipation-related symptoms.
- Once-daily administration of elobixibat for 14 days was found to be safe and well tolerated up to 20 mg in female and male patients with chronic constipation.

Introduction

Chronic constipation (CC) is one of the most common bowel disorders, with symptoms of infrequent stool movements, lumpy/hard stool, straining and a sense of incomplete evacuation [1]. Systematic reviews found a predominance of women and elderly people with CC [2, 3] and reported prevalence rates of 17.1% and 15.3% in Europe and Oceania, respectively [2]. In an online survey of 5155 Japanese, 28.4% respondents self-reported constipation [4]. Constipated individuals experience a physically and mentally lower quality of life [5] and a pose to greater socioeconomic burden [6] than nonconstipated individuals. Despite the high prevalence and availability of medications to treat CC, satisfaction levels with traditional treatments are not high. A web-based survey in the USA revealed that nearly half of respondents were not completely satisfied with their treatments [7]. These findings highlight the necessity of an emerging treatment approach that targets the multiple symptoms of CC.

Bile acids (BAs) in the gastrointestinal (GI) tract have a dual action as: (i) modulation of fluid and electrolyte absorption [8, 9]; and (ii) regulation of GI motility [10, 11]. **Elobixibat** (rINN) is a highly selective inhibitor of an **ileal BA transporter (IBAT)**, leading to augmentation of BA levels in the colon and subsequently enhancing colonic motility and secretion [12]. In addition, reduced ileal BA reabsorption upregulates hepatic BA synthesis from cholesterol and induces expression of low-density lipoprotein cholesterol (LDL-C) receptors on hepatocytes [13]. Thus, elobixibat decreases the plasma LDL-C level. Concurrently, elobixibat increases the level of the intermediate product of BA synthesis: plasma 7α-hydroxy-4-cholesten-3-one (C4), which is a surrogate of the hepatic BA synthesis rate [14, 15]. Another factor likely to increase BA synthesis is food intake, which may influence the pharmacological action of elobixibat.

To date, clinical trials conducted in the USA and Sweden have revealed the efficacy and safety of elobixibat with chronic idiopathic constipation [16–18]; however, few studies recruited both sexes, and the tolerability of a relatively high dose of elobixibat in male patients is unknown. Here, our study was carried out to initially assess the pharmacokinetics (PK), pharmacodynamics (PD), tolerability and efficacy of elobixibat in Japanese female and male patients with CC. We also examined the food effect and influence of sex on the pharmacological action in a single- and multiple-dose escalation study.

Methods

Study design: randomization and protocol

This study consisted of two major tests: a *single* administration test (S-test) and a *multiple* administration test (M-test) as described in Figure 1. Both tests were randomized, placebo-controlled, double-blind studies conducted at Kitasato University East Hospital (Kanagawa, Japan) between April 2013 and October 2013. The study was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice guidelines. Written informed consent was obtained from all patients before participation. The local Institutional Review Board at Kitasato University East Hospital approved the study protocol and the informed consent form (No. 2012012). The sponsor, all randomized patients and study centre personnel were blinded to study treatment allocation.

The S-test was a crossover, dose-escalating design also employed to clarify the food effect on the pharmacological action of elobixibat. Eligible patients were randomized in a 5:1 ratio to receive one of five dose levels of elobixibat ($n = 10$) or the corresponding placebo (n = 2). Test tablets were taken orally 15 min before breakfast or without breakfast (fasting in the morning). This is because elobixibat acts locally in the terminal ileum and should be taken before BA secretion induced by food intake for the retention effects. The different dose levels of elobixibat were 2.5, 5, 10, 15 and 20 mg. Intervention 1 was to receive the test tablets before breakfast or without breakfast on Day 1 (on the day of administration) followed by a 6-day washout (observation) period, and intervention 2 was to take the tablets on Day 8 with the alternative regimen followed by a 5-day observation period (Figure 1, left). All eligible patients were admitted to the study site 2 days before administration (Day –2) until Day 14 (on the day of discharge).

Similarly, eligible patients were randomized to receive one of five dose levels ($n = 10$) or the corresponding placebo ($n = 2$) in the M-test. Test tablets were taken orally once daily before breakfast for 14 consecutive days. The dose levels of elobixibat were the same as those in the S-test. All eligible

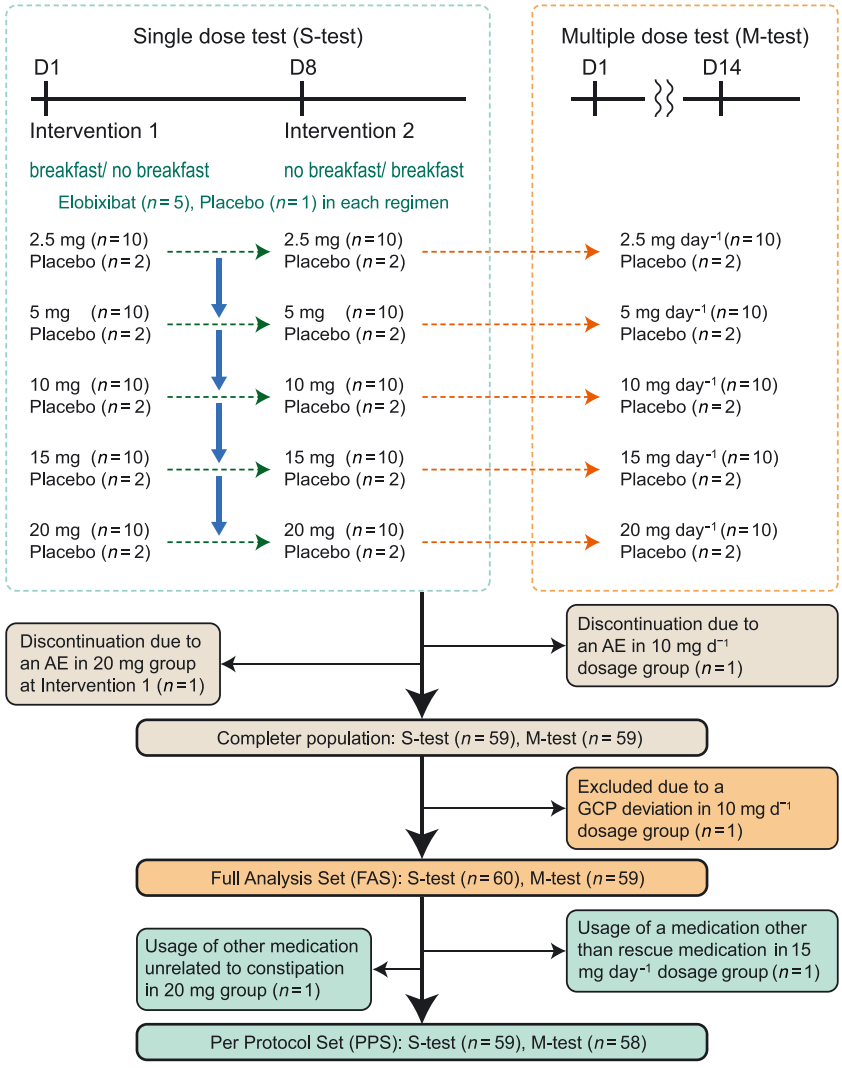

Figure 1

Study design and patient flow. Both the single-dose test (S-test) and the multiple-dose test (M-test) were dose-escalating studies and were conducted sequentially. The S-test was a crossover design in which a single dose of elobixibat or placebo was administered either before breakfast or without breakfast on Day (D) 1 (intervention 1) and D8 (intervention 2). In the M-test, a test tablet was administered once daily before breakfast for 14 consecutive days. Blue arrow indicates each safety assessment performed to determine dose escalation

patients were admitted to the study site on Day –2, followed by the 14-day treatment period and 6-day observation period, and then discharged on Day 21 (Figure 1, right).

The S-test and M-test were conducted sequentially, and each dose escalation was determined by the investigator's safety evaluation. A rescue medication (bisacodyl suppositories) was permitted if the patient had no bowel movement (BM) for 72 h or longer and had symptoms requiring further intervention.

Study participants

Eligible patients were men or nonpregnant and nonlactating women, 20–64 years of age, body mass index ≥18.5 and < 30.0 kg m^{-2}, with CC of at least 6 months' duration. The diagnosis of CC was defined as a history of fewer than three spontaneous BMs (SBMs) per week, with at least one of the following symptoms: (1) straining during ≥25% of BMs, (2) lumpy or hard stools during ≥25% of BMs and (3) sensation of incomplete evacuation during ≥25% of BMs. An eligible patient should not have had more than five SBMs during the 14 days before drug administration. The exclusion criteria included organic constipation, neurological constipation, constipation caused by hormonal imbalance, drug-induced constipation, history of GI obstruction, intestinal/rectal prolapse and intestinal/rectal resection except for simple appendectomy.

PK and PD analytical methods

Blood samples were routinely collected for the S-test every day from Day –1 throughout the study period of each arm (until Day 14) and were collected for the M-test on Days –1, 1, 2, 5, 7–9, 11, 13–16, 18 and 21. We additionally collected

blood samples at shorter intervals of pre dose (0.0), 0.5, 1, 1.5, 2, 3, 6, 8 and 12 h on Days –1, 1, 7 and 8 for the S-test and on Days – 1, 1, 8 and 14 for the M-test. Urine samples were collected every day from pre dose (0.0) throughout the study period of each arm, except Day 7 for the S-test. Plasma and urinary concentrations of elobixibat were measured for PK analysis using a liquid chromatography–tandem mass spectrometry (LC–MS/MS) with a lower limit of quantification (LLOQ) of 10.0 pg ml^{-1} by LSI Medience Corporation (Tokyo, Japan). The serum LDL-C, serum high-density lipoprotein cholesterol (HDL-C) and plasma C4 levels were measured for PD analysis by Shin Nippon Biomedical Laboratories, Ltd. (SNBL, ltd., Wakayama, Japan). C4 was measured using LC–MS/MS with an LLOQ of 2 ng ml^{-1}, and cholesterol was measured by the ultracentrifugation method with an LLOQ of 2 mg dl^{-1} for LDL-C and an LLOQ of 5 mg dl^{-1} for HDL-C. Within the C4 measurements, the interbatch precision was between 1.5% and 5.2%, whereas the interbatch relative error was in the range of −0.6–10.0% of nominal concentration.

Efficacy assessments

Given that this was an exploratory study, we did not set a primary outcome measure. The following information was recorded for each patient: the time of each BM (as BM, SBM or complete SBM), stool consistency according to the Bristol Stool Form Scale [19] (seven levels), straining conditions (five levels), completeness of evacuation (Yes/No), GI symptoms [bloating (five levels), discomfort (five levels), severity of constipation (five levels) and abdominal pain (Yes/No)]. Efficacy assessment also included the use of rescue medication.

Safety assessments

Safety evaluation included the type, severity and incidence of adverse events (AE), physical examinations, vital signs (pulse, blood pressure, body temperature and weight), electrocardiography [standard 12-lead echocardiography (ECG; Cardico1211; SUZUKEN Co., Ltd., Nagoya, Japan)], and laboratory tests (haematology, biochemistry and urinalysis). QT intervals on ECG were also measured using automated reading.

Data analysis and statistical methods

This study was exploratory in nature. Descriptive statistics were provided for all PK, PD, demographic, efficacy and safety parameters. Phoenix WinNonlin 6.1 (Certara L.P., Princeton, NJ, USA) was used to estimate PK and PD parameters by noncompartment model analysis. The area under the concentration–time curve (AUC) was estimated via linear trapezoidal rule. The changes in AUC of PD markers at each time point from baseline (Day –1) were calculated as areas under the effect curve (AUEC), and the maximum effect (E_{max}) from baseline were also measured. All statistical analyses were performed using SAS 9.1.3 SP4 (SAS Institute Inc., NC, USA) by ASKLEP Inc. (Tokyo, Japan). Statistical tests of significance were two-sided, and the significance level was set at $\alpha = 0.05$.

PK-dose proportionality in the no-breakfast regimen was assessed using a power model or analysis of variance (ANOVA). Log-transformed PK values were used for analyses, and back transformation was applied when appropriate. Accumulation ratios (R_{ac}) for maximum plasma concentration (C_{max}), $AUC_{(0-t)}$, terminal half-life ($t_{1/2}$) and time to C_{max} (t_{max}) in the M-test were calculated as the ratios of the respective values on Day 8 over Day 1 and Day 14 over Day 1. Analysis of covariance (ANCOVA) was used to estimate least squares means (LSMs) and 90% confidence intervals (CIs) of every dosage arm from pooled elobixibat groups.

Food effects on PK values as well as AUEC of each PD marker were investigated with a linear mixed effect model (LME) that included dose, food (breakfast or no breakfast), intervention (1 or 2; see Figure 1) and period of breakfast consumption (first batch or second batch) as fixed effects, and patient as the random effect. The effects of elobixibat on PD markers were evaluated using ANCOVA, including baseline value (Day –1) and dose as covariants. The relationship between elobixibat plasma concentration and each PD parameter was analysed using spaghetti plotting.

The correlation between C4 AUEC and the number of SBMs as well as the correlation between stool consistency and SBM frequency were assessed by Pearson correlation coefficient.

We used ANCOVA to clarify if sex influences C_{max} and $AUC_{(0-t)}$, AUEC of each PD marker and SBM frequency.

Safety assessments were performed by evaluating the number/proportions of patients experiencing AEs and by analysis of any changes from baseline in laboratory tests, ECG and vital signs. The dose-dependency of AEs was analysed using the Cochran–Armitage trend test.

Following the ICH-E14 guideline, a 12-lead surface ECG was used for general safety evaluation in addition to the Fridericia-corrected QT intervals (QTcF) measured by experienced readers operating from a centralized ECG laboratory (BioClinica, Inc, PA, USA). The changes in QTcF from baseline at each time point (ΔQTcF) were calculated, then a point estimate (ΔΔQTcF) and the 90% CIs were estimated between elobixibat and placebo groups. The number and proportion of patients with absolute QTcF (>450 ms, >480 ms and > 500 ms) and ΔQTcF (>30 ms and > 60 ms) were summarized for a categorical analysis.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [20] and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [21].

Results

Participant flow

Of 498 CC patients screened, 120 patients including 59 men and 61 women were enrolled in this study. Two patients were withdrawn due to AEs; hence, 118 completed the study. One patient, who was found to have an offence against the Good Clinical Practice guidelines after the completion of the study, was excluded from all analyses. Therefore, 60 patients in the S-test and 59 patients in the M-test were included in the full analysis set, which was used for the PK, PD, PK/PD and safety

analyses, but the placebo group was not included in the PK and PK/PD analyses. The per protocol set was used for efficacy analysis. Two patients were excluded from the per protocol set: one patient in the S-test who had a non-constipation-related symptom (dermatitis) requiring medication and another patient in the M-test who used a medication that was not a rescue drug (Figure 1). The study groups were well balanced, and all treatment groups had female and male patients with generally comparable characteristics (mean ± standard deviation): age (35.4 ± 10.8 years), height (165.9 ± 7.7 m), weight (61.1 ± 9.0 kg), body mass index (22.2 ± 2.5 kg m^{-2}) and baseline (Week –1) SBM frequency (1.7 ± 0.7). The complete CONSORT patient flow and baseline demographics are shown in Figure S1 and Table S1 of the online supporting documents.

PK-dose proportionality and drug accumulation

Both C_{max} and AUC increased dose-dependently, even though the plasma elobixibat concentration was exiguous (in the picomolar range). Moreover, elobixibat was scarcely excreted in the urine. The fraction of dose excreted in urine (fe) up to 144 h in the no-breakfast regimen was <0.01% in all dosing groups (Table 1).

Table 1

Selected plasma elobixibat pharmacokinetic parameters

	Elobixibat dose				
	2.5 mg	**5 mg**	**10 mg**	**15 mg**	**20 mg**
Single administration					
no breakfast					
n	10	10	10	10	9
C_{max} (pg ml^{-1})	413.05 (66.4)	582.89 (40.8)	1357.49 (65.0)	1807.20 (45.7)	3165.49 (54.1)
$AUC_{(0-t)}$ (pg h ml^{-1})	1662.60 (53.1)	2732.12 (33.3)	5462.58 (60.2)	7999.17 (42.1)	12839.38 (40.9)
$t_{1/2}$ (h)	2.17 (39.3)	3.94 (59.3)	5.69 (62.7)	9.90 (94.7)	11.53 (44.5)
Ae_{144h} (ng)	213.28 (59.5)	306.59 (38.8)	598.14 (67.8)	873.09 (68.4)[a]	1623.23 (41.5)
fe_{144h} (%)	0.0098	0.0077	0.0070	0.0070[a]	0.0088
before breakfast					
n	10	10	10	10	10
C_{max} (pg ml^{-1})	101.10 (90.6)	170.34 (46.6)	343.64 (55.8)	376.80 (26.6)	691.92 (76.7)
$AUC_{(0-t)}$ (pg h ml^{-1})	227.43 (59.7)	633.28 (61.7)	1086.53 (52.9)	1506.79 (29.3)	2940.11 (71.5)
$t_{1/2}$ (h)	1.62 (70.2)[b]	2.64 (93.6)	2.22 (61.5)	2.98 (46.6)	4.18 (96.7)
Ae_{144h} (ng)	24.30 (72.9)[a]	107.50 (63.4)	139.38 (49.9)	188.43 (37.2)[a]	384.85 (55.6)[a]
fe_{144h} (%)	0.0008[a]	0.0021	0.0016	0.0013[a]	0.0021[a]
Multiple administration					
n	10	10	8	10	10
C_{1max} (pg ml^{-1})	98.69 (56.9)	164.83 (54.7)	284.67 (39.6)[a]	468.71 (63.7)	932.04 (76.3)
C_{8max} (pg ml^{-1})	99.39 (63.6)	139.45 (50.7)	283.84 (17.9)	388.55 (82.0)	581.32 (53.6)
C_{14max} (pg ml^{-1})	82.66 (50.4)	165.57 (35.0)	236.11 (34.4)	383.19 (73.5)	953.18 (53.6)
$AUC_{1(0-t)}$ (pg h ml^{-1})	313.58 (30.5)	558.58 (42.5)	1125.23 (27.1)[a]	1751.43 (53.7)	3024.88 (50.6)
$AUC_{14(0-t)}$ (pg h ml^{-1})	248.66 (27.0)	750.54 (28.0)	1355.22 (33.8)	1968.18 (47.0)	3445.62 (31.5)
$t_{1(1/2)}$ (h)	1.89 (40.9)[a]	2.41 (32.7)	2.69 (30.7)[a]	3.28 (75.7)	3.14 (62.4)
$t_{8(1/2)}$ (h)	2.42 (31.2)	3.29 (68.9)	4.96 (64.1)	4.62 (58.2)	5.16 (45.2)
$t_{14(1/2)}$ (h)	2.34 (50.8)[a]	3.73 (61.0)	6.19 (146.0)	5.68 (74.9)	7.23 (37.5)

Selected pharmacokinetic parameters, presented as geometric mean (coefficient of variance), except for fe_{144h}, which is presented as arithmetic mean

[a] $n = 9$;

[b] $n = 8$.

Ae_{144h}, cumulative amount of drug excreted unchanged in urine from zero to 144 h; $AUC_{(0-t)}$, area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration; C_{max}, maximum plasma concentration; fe_{144h}, fraction of dose excreted unchanged into urine from zero to 144 h; $t_{1/2}$, terminal half-life. C_{1max}, C_{8max} and C_{14max} indicate C_{max} on Day 1, 8 and 14, respectively, and corresponding numbers are used for $AUC_{(0-t)}$ and $t_{1/2}$

The analysis fitted to the power model estimated the slopes of the regression lines in the no-breakfast regimen to be 0.9618 (95% CIs: 0.7161–1.2076) for C_{max}, 0.9465 (95% CIs: 0.7579–1.1351) for AUC_{∞} and 0.9321 (95% CIs: 0.6719–1.1923) for cumulative amount of drug excreted in urine from zero to 144 h (Ae_{144h}), suggesting dose proportionality of the PK parameters. Meanwhile, $t_{1/2}$ was prolonged dose-dependently ($P < 0.001$). This is very likely to be an apparent prolongation as plasma elobixibat concentration fell below the LLOQ earlier at lower doses.

The plasma elobixibat concentration with multiple administration reached a steady state by Day 8 (Table 1). No drug accumulation was observed from the C_{max} in all dosage groups, as the 90% CIs of Rac included 1.000. Meanwhile, slight drug accumulation was observed from $AUC_{(0\text{-}t)}$ on Day 14 in 5-mg day^{-1} dosage (data not shown).

The effects of elobixibat on C4, HDL-C and LDL-C levels in the M-test

Plasma C4 levels in all elobixibat groups increased from Day 1 and continued to increase throughout the multiple administration period (Table S2). The E_{max} and AUEC showed dose-dependent augmentations with a plateau at the 15-mg day^{-1} dosage, and a maximum 9– 10-fold increase in E_{max} was observed relative to placebo (Figure 2A). The ANCOVA found that C4 AUEC on both Day 8 and Day 14 were significantly greater in the elobixibat groups relative to placebo

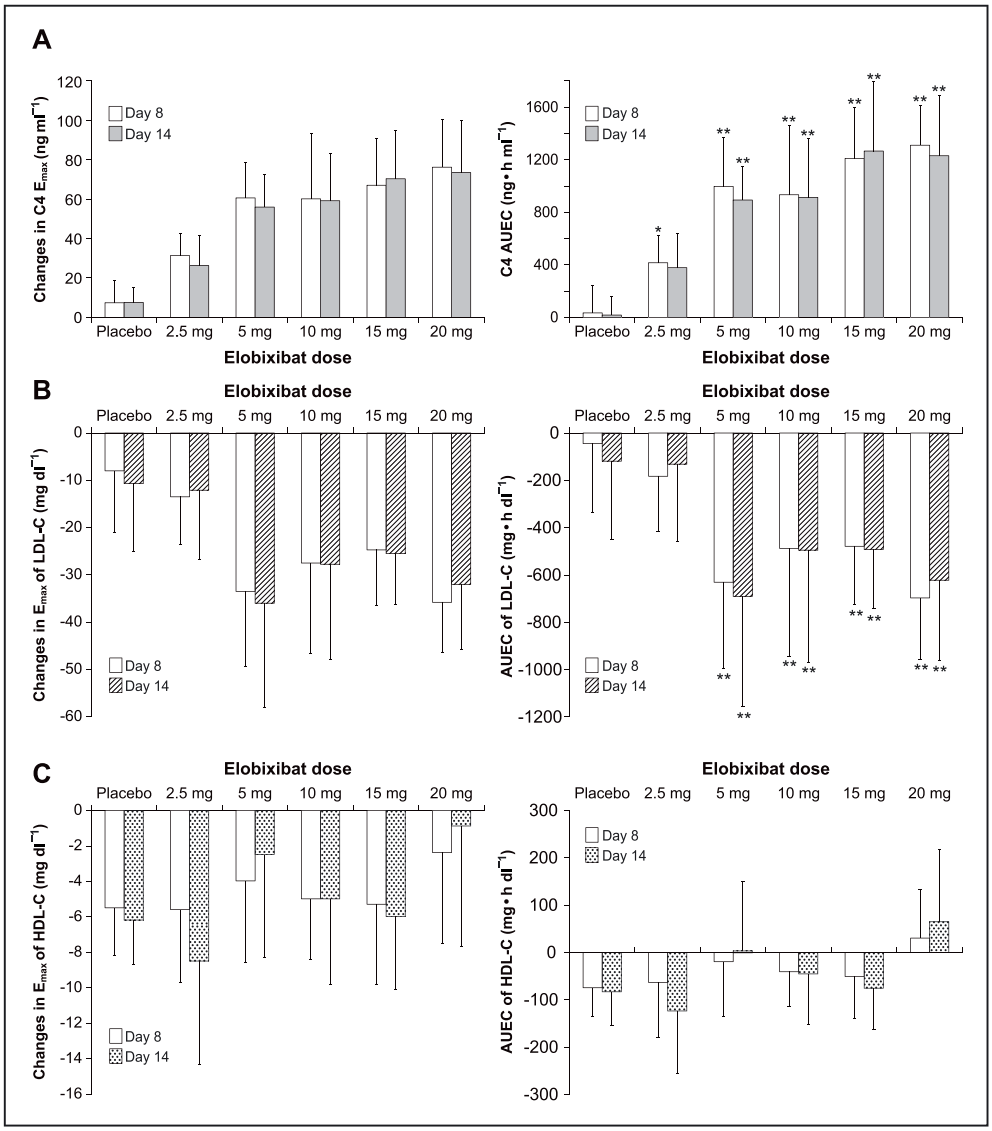

Figure 2

Dose-dependent increases of E_{max} and AUEC in C4 (A), reductions in LDL-C (B) from baseline, and no dose-dependency in HDL-C (C) in the M-test. Each bar and error bar indicate mean and standard deviation. Analysis of covariance (ANCOVA) found significant differences in AUEC of C4 and LDL-C between elobixibat and placebo groups on both Day 8 and Day 14 (*$P < 0.05$, **$P < 0.001$). AUEC, area under the effect curve; E_{max}, maximum effect; C4, 7α-hydroxy-4-cholesten-3-one; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

($P < 0.001$ except 2.5 mg day^{-1}). In contrast, mean serum LDL-C concentration in the elobixibat groups continued to decrease during the administration period in all except 2.5 mg day^{-1} (Table S2). The E_{max} and AUEC of LDL-C showed reductions in the elobixibat groups with a plateau at 5 mg day^{-1}, and a maximum 3– 4-fold decrease in E_{max} was observed relative to placebo (Figure 2B). The ANCOVA demonstrated that the AUEC of LDL-C on both Day 8 and Day 14 showed significantly greater reductions in the elobixibat groups compared to placebo ($P < 0.001$ except 2.5 mg day^{-1}). There was no clear trend for HDL-C levels (Table S2); hence, no dose-dependency on the AUEC or E_{max} was observed (Figure 2C).

Moreover, PK/PD analysis indicated that the plasma C4, serum LDL-C and HDL-C levels were independent of plasma elobixibat concentration at all dosages (data not shown).

Food effects in the single administration test

The plasma elobixibat concentration after breakfast was much lower than in the fasted state, and there was no major difference in $t_{1/2}$ (Figure 3). Table 2 shows the LSMs of the inverse log-transformed PK values (breakfast/no breakfast) and the 90% CIs. The analysis indicated that breakfast consumption reduced exposure by approximately 80% relative to fasting at every dosing group. In contrast, both C4 E_{max} and AUEC in the breakfast regimen showed significantly greater elevations compared with the no-breakfast regimen (AUEC: $P < 0.001$; Figure 4).

Sex-based differences

There were no sex-specific effects on C_{max} and $AUC_{(0–t)}$ analysed with LME (Table 3). The analyses fitted ANCOVA found no differences between female and male in C4 AUEC, LDL-C AUEC or the number of SBMs (data not shown).

Table 2

Food effects analysed using linear mixed effect model

Breakfast/no breakfast		Back transformed value		
			90% CI	
PK parameters	dose	LSM	lower bound	upper bound
C_{max} (pg ml^{-1})	2.5 mg	0.2448	0.1786	0.3354
	5 mg	0.2922	0.2265	0.3770
	10 mg	0.2531	0.1574	0.4072
	15 mg	0.2085	0.1371	0.3172
	20 mg	0.2240	0.1411	0.3558
$AUC_{(0–t)}$ (pg h ml^{-1})	2.5 mg	0.1368	0.1111	0.1685
	5 mg	0.2318	0.1756	0.3060
	10 mg	0.1989	0.1560	0.2536
	15 mg	0.1884	0.1407	0.2521
	20 mg	0.2275	0.1458	0.3551

Breakfast consumption reduced the maximum plasma concentration (C_{max}) and area under the concentration–time curve [$AUC_{(0–t)}$] by approximately 80% compared with the no-breakfast regimen, which was confirmed by back-transformed LSM (the ratio of breakfast/no breakfast)
LSM, least squares mean; CI, confidence interval

Bowel functions in the multiple administration test

The changes in SBM frequency from baseline (Week –1) showed dose-dependent increases, which were similar at Weeks 1 and 2 in all dosage groups (Figure 5). We also analysed the relationship between C4 levels and SBM frequency. The squared Pearson correlation coefficients (R^2) in Weeks 1 and 2 were 0.4973 and 0.5929, respectively, suggesting positive correlations (Figure 6).

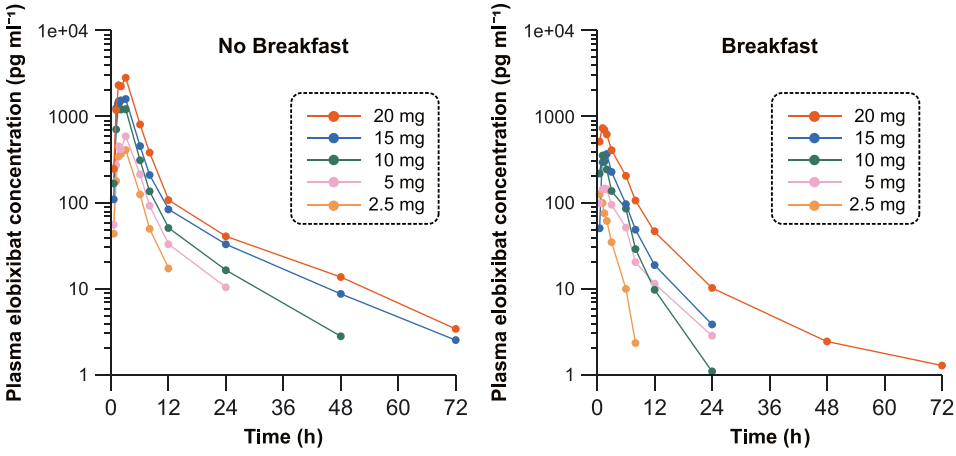

Figure 3

Plasma elobixibat concentration-time profile after single administration of elobixibat taken without breakfast (left) or before breakfast (right) in the S-test. Reductions of plasma concentration with breakfast consumption were observed. Mean values of log-transformed concentration are indicated in each dosing group

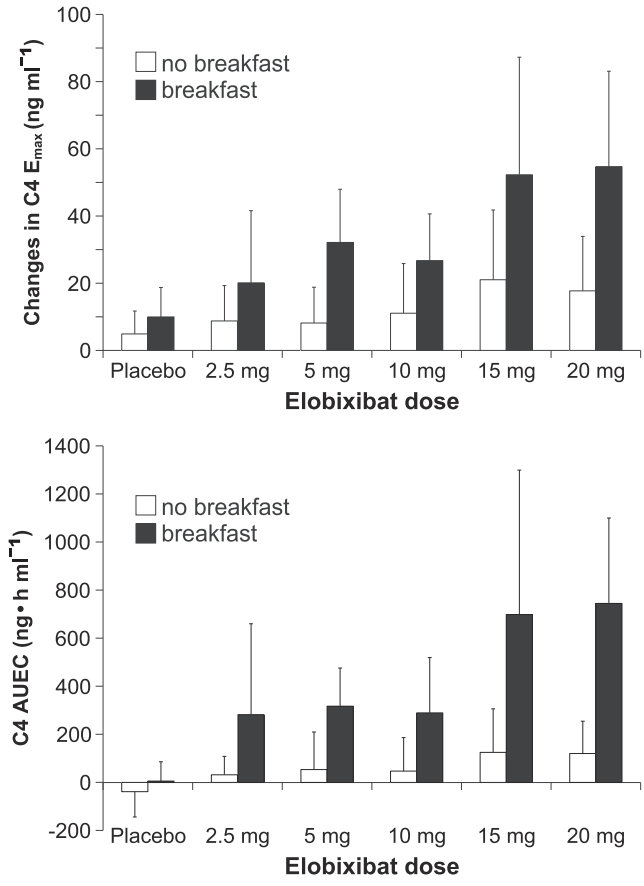

Figure 4

Food effect on C4 E_{max} and AUEC in the S-test. Each bar and error bar indicate mean and standard deviation. The data were obtained up to 24 h after elobixibat administration. A significant difference was noted between breakfast and no-breakfast regimens from pooled elobixibat groups compared with placebo ($P < 0.001$). There were also significant differences between regimens in each elobixibat group

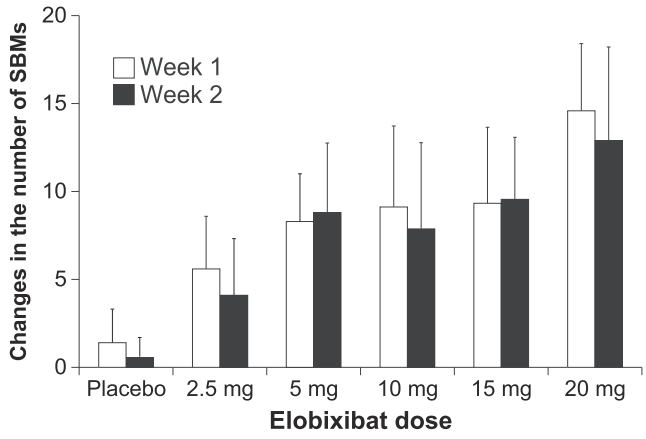

Figure 5

Dose-dependent increase in changes in spontaneous bowel movement (SBM) frequency from baseline (Week –1) in the M-test. Each bar and error bar indicate mean and standard deviation

Most of the patients had a first SBM within 24 h after elobixibat administration. The proportions (number of patients) were 40.0% (4/10) in the placebo; 100% (10/10) in the 2.5-, 5- and 20-mg day^{-1}; 100% (9/9) in the 10-mg day^{-1} and 88.9% (8/9) in the 15-mg day^{-1} group. The time to the first SBM was evidently shorter in the elobixibat groups than in the placebo group.

Other bowel functions were improved in all elobixibat groups: stool consistency, severity of constipation, sense of incomplete evacuation and straining conditions. Bloating was improved at ≥5-mg day^{-1} group in 50–90% of patients. Nearly half of the patients in the 5- and 10-mg day^{-1} groups reported improvement of abdominal discomfort, while 40% of patients in the 20-mg day^{-1} group reported worse abdominal discomfort. In the elobixibat groups, 30–60% of patients reported worsened abdominal pains relative to 0% in the placebo group.

There were also positive correlations between stool consistency and SBM frequency in the 10-mg day^{-1} ($R^2 = 0.7813$), 15-mg day^{-1} ($R^2 = 0.6949$) and 20-mg day^{-1} ($R^2 = 0.6424$) groups (Figure S2). No patient in the study used rescue medication.

Safety assessments

The common GI-related AEs reported in both the S-test and M-test are summarized in Table 4. One patient in the single

Table 3

Sex-based differences analysed with the linear mixed effect model

Male/female PK parameters	Back transformed value			
		95% CI		
	LSM	lower bound	upper bound	*P* value
C_{8max} (pg ml^{-1})	0.8416	0.6264	1.1306	0.2451
C_{14max} (pg ml^{-1})	0.9675	0.7203	1.2996	0.8225
$AUC_{8(0-t)}$ (pg h ml^{-1})	0.9947	0.8026	1.2329	0.9606
$AUC_{14(0-t)}$ (pg h ml^{-1})	0.9863	0.8157	1.1925	0.8838

There were no differences between female and male in maximum plasma concentration (C_{max}) and area under the concentration–time curve [$AUC_{(0-t)}$]
LSM, least squares mean; CI, confidence interval

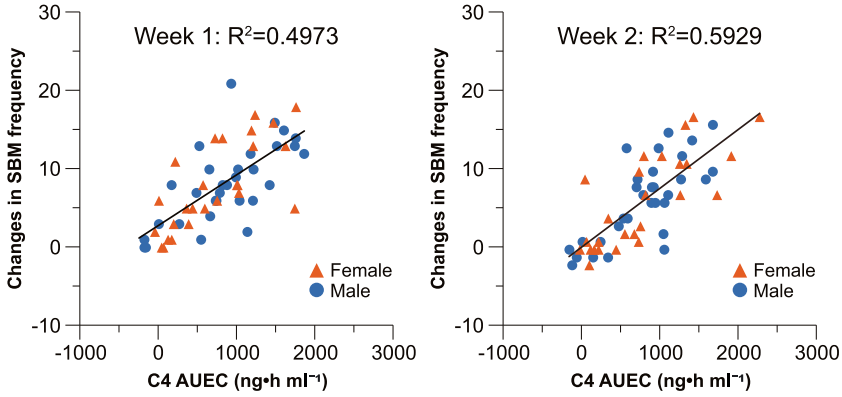

Figure 6

Scatter plots showing the relationship between changes in spontaneous bowel movement (SBM) frequency from baseline and C4 area under the effect curve in the M-test. The coefficient of determination (R^2) was calculated, and positive correlations are indicated in Weeks 1 and 2

20-mg and another in the multiple 10-mg day^{-1} group were withdrawn due to AEs of headache and urticaria, respectively (Figure 1). The AEs reported in the S-test were mainly GI symptoms, and all AEs were mild and resolved. There was no relationship between dose and number of AEs. GI symptoms such as bloating, lower abdominal pain and diarrhoea were frequently experienced in the elobixibat groups. Those symptoms were probably due to the pharmacological effects of elobixibat; nevertheless, the frequency of GI symptoms showed no dose-dependency (P = 0.6037, S-test).

Similarly, GI symptoms were mainly reported in the M-test. The number of GI-related AEs was more in the elobixibat groups than in the placebo but showed no dose-dependency (P = 0.1015, M-test). One patient in each of the 10- and 20-mg day^{-1} groups experienced moderate lower abdominal pains, although those conditions resolved without any treatment. The remaining AEs reported in the M-test were all mild and most symptoms were spontaneously resolved.

Neither prolongation of QTcF intervals nor dose-dependent prolongation of ΔΔQTcF was observed in this whole study. Moreover, within the categorical analysis, no QTcF prolongation (>450 ms) was observed.

Discussion

This study provided supporting evidence that elobixibat should be taken before breakfast. Our study also revealed a dose-dependent increase in SBM frequency with corresponding elevation of plasma C4 levels, and elobixibat is safe and tolerable up to 20 mg.

The plasma elobixibat concentration after single oral administration was in the picomolar range and extremely low. When 2.5–20 mg of elobixibat was taken in a fasted state; however, the dose proportionality with regards to C_{max} and AUC was indicated by a power model. In contrast, breakfast consumption (15 min after medication) reduced elobixibat concentration by 80% compared with fasting. In addition, the quantity of drug excreted in the urine was less than 0.01% at all dosing levels in both regimens. Likewise, multiple administration scarcely increased the plasma concentration, and no obvious drug accumulation was observed other than slight accumulation at the 5- and 15-mg day^{-1} dosages.

Elobixibat should be administered before secretion of BAs into the duodenum for the maximum effect. To confirm this, we have carried out a crossover study with and without breakfast. When elobixibat was taken before breakfast, plasma C4 level was raised while the C_{max} and AUC were declined compared with fasting. This result indicates that BA synthesis was upregulated by food intake as well as due to interruption of enterohepatic circulation [12, 22] by elobixibat. However, we did not observe a major difference in the incidence of GI-related AEs between regimens. With this enhancement of pharmacological action by food consumption, and safety concerns over the incidence of unexpected AEs due to relatively high plasma concentrations in the fasting state, it is ideal that elobixibat be taken before breakfast.

Increased SBM frequency had a good correlation with higher C4 levels, which was in line with reports from a previous clinical trial [16]. The reduction of LDL-C level plateaued at the 5-mg dosage, while the elevation in C4 levels saturated at the 15-mg dosage. This difference could be because a certain number of LDL-C binding events are required prior to BA synthesis.

There was a dose–response relationship with SBM frequency, as well as a positive correlation between SBM frequency and plasma C4 levels. As C4 is an intermediate product of BA synthesis, the elevation of C4 is most likely to be associated with improvement of GI motility. Therefore, plasma C4 levels can be an excellent biomarker for the effect of elobixibat.

The number of SBMs increased at the minimum dosage (2.5 mg) explored in this study. Moreover, most of the patients in the elobixibat groups had a first SBM within 24 h, and improved other bowel functions regardless of the dose received. Thus, these results suggested that elobixibat is effective at a low dose. The one of the main action of BA – increasing fluid – rapidly improved other bowel functions such as stool consistency, severity of constipation and a sense of incomplete evacuation. Another action of BA – enhancing motility – dose-dependently improved SBM frequency. This was also supported by our results that higher C4 levels were correlated with increased SBM frequency.

Table 4

Gastrointestinal adverse events experienced by two or more patients in any group

System organ class Preferred term	Placebo		Elobixibat dose 2.5 mg		5 mg		10 mg		15 mg		20 mg	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Single administration												
no breakfast												
n	10		10		10		10		10		9	
Any AEs	4	(40.0)	4	(40.0)	5	(50.0)	3	(30.0)	10	(100.0)	4	(44.4)
AEs associated with gastrointestinal disorders	3	(30.0)	2	(20.0)	5	(50.0)	1	(10.0)	10	(100.0)	1	(11.1)
abdominal distention	1	(10.0)	0		2	(20.0)	0		2	(20.0)	1	(11.1)
abdominal pain lower	1	(10.0)	1	(10.0)	2	(20.0)	1	(10.0)	3	(30.0)	0	
diarrhoea	0		0		3	(30.0)	0		7	(70.0)	0	
before breakfast												
n	10		10		10		10		10		10	
Any AEs	7	(70.0)	4	(40.0)	8	(80.0)	2	(20.0)	6	(60.0)	4	(40.0)
AEs associated with gastrointestinal disorders	5	(50.0)	4	(40.0)	8	(80.0)	1	(10.0)	6	(60.0)	3	(30.0)
abdominal distention	2	(20.0)	2	(20.0)	0		0		1	(10.0)	2	(20.0)
abdominal pain lower	3	(30.0)	2	(20.0)	4	(40.0)	1	(10.0)	1	(10.0)	1	(10.0)
diarrhoea	1	(10.0)	0		4	(40.0)	0		5	(50.0)	0	
Multiple administration												
n	10		10		10		9		10		10	
Any AEs	6	(60.0)	8	(80.0)	9	(90.0)	9	(100.0)	9	(90.0)	9	(90.0)
AEs associated with gastrointestinal disorders	4	(40.0)	8	(80.0)	8	(80.0)	7	(77.8)	7	(70.0)	9	(90.0)
diarrhoea	0		1	(10.0)	4	(40.0)	4	(44.4)	4	(40.0)	8	(80.0)
abdominal distention	3	(30.0)	3	(30.0)	3	(30.0)	2	(22.2)	4	(40.0)	6	(60.0)
abdominal pain lower	2	(20.0)	4	(40.0)	3	(30.0)	3	(33.3)	4	(40.0)	5	(50.0)
abdominal pain upper	0		0		2	(20.0)	2	(22.2)	0		2	(20.0)

AE, adverse event
MedDRA version 16.0 was used for medical terminology

Elobixibat was well tolerated up to 20 mg in both the S-test and the M-test as most of AEs were mild and resolved. The main AEs were GI symptoms, which were expected due to the localized action of elobixibat. The number of GI-related AEs did not show any dose-dependency after single administration, as the incidence was the highest at the 15-mg dosing in the no-breakfast regimen and at the 5-mg dosing in the breakfast regimen. Likewise, the Cochran–Armitage trend test did not show a dose-dependency of the number of GI-related AEs in the M-test, possibly because a similar number of GI symptoms was observed at each dosage group. Nevertheless, a positive correlation was observed between SBM frequency and stool conditions. Multiple 20-mg day^{-1} dosage increased the number of SBMs but had an undesired liquifying effect on the stools, which led to diarrhoea (see Figure S2 and Table 4). A larger-phase study with multiple dosing will be needed to clarify the dose-dependency of the incidence of GI-related AEs.

No clinically serious AE associated with laboratory measurements was reported other than slight and transient changes in the measurements. No significant changes in ΔΔQTcF were found at any dosing, and no QTcF prolongation was observed in the categorical analysis. Therefore, it is unlikely that the elobixibat doses explored in this study have a risk of QT prolongation. These results also suggest that elobixibat up to 20 mg is safe and tolerable.

The limitation of this study is its small sample size (10 patients per arm) used to evaluate the pharmacological actions of elobixibat. It is impossible to draw a definite conclusion and apply those data to all patients with CC.

Nonetheless, our study was conducted under well-controlled inpatient settings, and demonstrated the efficacy and safety of elobixibat. We also found that sex does not influence plasma elobixibat concentration, plasma C4 and serum LDL-C levels or SBM frequency. This is the first study to demonstrate that 20-mg elobixibat is tolerated in both female and male patients. These results provide support for the therapeutic use of elobixibat in both female and male patients with CC. Large-scale, long-term studies are required to select an appropriate dosage and to evaluate the efficacy and safety of long-term treatment with elobixibat.

In summary, the picomolar concentration of elobixibat in plasma observed in our PK study indicated minimal systemic bioavailability. We also found that elobixibat increased BA synthesis dose-dependently, and concurrently increased SBM frequency. Elobixibat also improved other bowel and abdominal symptoms in female and male patients with CC.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). I.O. is an employee of EA Pharma Co., Ltd. H.F. has received consultancy fees from EA Pharma Co., Ltd. Y.K., H.A., Y.S., C.N. and M.M. have no competing interests to declare.

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Contributors

Y.K., Y.S., C.N., M.M., I.O. and H.F. designed and, together with H.A., carried out the study. H.A. participated in data acquisition and analysis. I.O. carried out the statistical analysis and, together with Y.K. and H.F., interpreted the data. Y.K. wrote the first draft and edited drafts with I.O. and H.F. All authors reviewed and approved the final draft of the manuscript for submission.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

http://onlinelibrary.wiley.com/doi/10.1111/bcp.13698/suppinfo

Figure S1 CONSORT patient flow
Figure S2 Scatter plots showing the relationship between the number of SBMs and stool consistency (Bristol Stool Form Scale) during Weeks 1 and 2 in the M-test
Table S1 Patient demographics and baseline characteristics shown as mean (SD)
Table S2 Changes in plasma C4 and serum cholesterol levels in the M-test