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Impact of elobixibat on serum and fecal bile acid levels and constipation symptoms in patients with chronic constipation

Atsushi Nakajima,* Sonoko Ishizaki,† Kazuki Matsuda,† Shinsuke Kurosu,† Shinya Taniguchi,† Per-Göran Gillberg,‡ Jan P Mattsson,‡ Tomoko Hasunuma§ and Michael Camilleri¶

*Department of Gastroenterology and Hepatology, Yokohama City University, Yokohama, †EA Pharma Co., Ltd., §Kitasato University Kitasato Institute Hospital, Tokyo, Japan; ‡Albireo AB, Gothenburg, Sweden; ¶Mayo Clinic, Rochester, Minnesota, USA

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Correspondence
Dr Atsushi Nakajima, Department of Gastroenterology and Hepatology, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
Email: nakajima-tky@umin.ac.jp

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Author contribution: AN, SI, ST, PG, and JM conceived and designed the study. TH acquired the data; KM and SK interpreted the data. AN and MC drafted the manuscript. All authors reviewed the manuscript drafts for important intellectual content and approved the final version of the manuscript for submission.

Abstract

Background and Aim: Elobixibat is a locally acting inhibitor of the ileal bile acid transporter. We compared bile acid metabolism between healthy subjects and patients with chronic constipation and assessed changes in the bile acid profile after elobixibat administration in the latter group.
Methods: Healthy subjects (n = 10) and patients with chronic constipation (n = 19) were assessed as inpatients for 7 days, during which they received meals containing ~60 g/day of fat. Patients with chronic constipation remained as inpatients for a further 7 days for once-daily elobixibat administration. Assessments included concentrations of fecal and serum bile acids, serum 7α-hydroxy-4-cholesten-3-one (C4) and fibroblast growth factor 19, and bowel movements and constipation symptoms.
Results: Fecal total and primary bile acids were significantly lower in patients with chronic constipation *versus* healthy subjects. Serum C4 and fibroblast growth factor 19 levels were comparable between groups. Elobixibat treatment increased fecal total and primary bile acids and decreased levels of fecal lithocholic acid and serum total as well as secondary bile acids in patients with chronic constipation. Bowel movements and other constipation-related symptoms were also improved by elobixibat to levels almost comparable with those of healthy subjects.
Conclusions: Despite comparable C4 levels, patients with chronic constipation demonstrated decreased levels of fecal bile acids *versus* healthy subjects. Elobixibat treatment increased fecal bile acid excretion and reduced serum bile acid concentrations. The improvement of constipation after elobixibat treatment was associated with increased total bile acids, particularly primary bile acids.

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Introduction

Bile acids (BAs) are physiological laxatives that stimulate colonic secretion[1] and motility.[2] Elobixibat (formerly A3309), a novel, locally acting inhibitor of the ileal BA transporter (IBAT),[3] prevents enterohepatic circulation of BAs, leading to colonic BA accumulation. It was approved in Japan for the treatment of chronic constipation in January 2018.

After being released into the duodenum, most BAs are actively reabsorbed via the IBAT and transported to the liver. Approximately 15% of conjugated BAs escape this process and enter the colon, where primary BAs (cholic acid [CA] and chenodeoxycholic acid [CDCA]) undergo deconjugation and dehydroxylation into secondary BAs (including deoxycholic acid [DCA] and lithocholic acid [LCA]). Additionally, ursodeoxycholic acid is produced by gut microbiota.[4,5] Deconjugated BAs are passively reabsorbed through the colonic mucosa; consequently, approximately 5% of BAs are fecally excreted.

Constipation is associated with reduced fecal or serum BAs in children[6] and adults.[7] A study of patients with

constipation-predominant irritable bowel syndrome (IBS) found that although ~15% displayed BA deficiencies compared with healthy controls, only 4.4% exhibited decreased fasting serum 7α-hydroxy-4-cholesten-3-one (C4, a precursor of BAs and surrogate marker for *de novo* BA synthesis).[8]

Bile acids are signaling molecules involved in lipid metabolism, electrolyte transport or colonic motility, and immune responses.[5,9] BAs can also modulate microbial balance in the gut, which alters the pool of BAs.[10] Considering this metabolic crosstalk between intestinal BAs and microbiota, characterization of BA profile modulation after elobixibat administration is warranted. Previous evaluation of BA composition in stools in response to elobixibat treatment was conducted in outpatients.[11] Given the day-to-day intrasubject variability and heterogeneity in the BA composition of a single stool, there are concerns about the validity of fecal sampling methods, particularly collection duration and control of dietary fat and fiber intake.[12,13] Notably, the results of a qualitative and quantitative study concluded that stool samples should be collected for at least 3 days, and ideally 5 days, to avoid errors.[14]

The role of BAs has been investigated in constipation-predominant IBS,[8] but not in other forms of chronic constipation; therefore, investigating the associations between types of constipation is important for understanding the clinical importance of elobixibat.

We conducted a study in participants admitted to a clinical research center that included provision of standard meals and a 6-day stool collection timescale. We compared serum and fecal BA metabolism between healthy subjects and patients with chronic constipation after elobixibat administration to investigate the change in BA profiles and to explore the accuracy of different stool sampling methods.

Materials and methods

Study design. This was an open-label, single-center study conducted between October 31, 2018 and October 30, 2019. Participants were recruited nationwide from the applicant pool for clinical research and were enrolled in the order their applications were received; remuneration for participation was provided.

After screening, all participants entered a run-in period, followed by a 1-week hospitalization (baseline period). After Day 7, healthy subjects left the center, while patients with chronic constipation remained for a further 7 days to receive elobixibat treatment. Oral elobixibat (10 mg) was administered 30 min before breakfast once daily; the dose could be up-/down-titrated as necessary (Fig. 1).

Participants underwent 10.5 h of fasting before admission. The center provided meals containing ~60 g of fat (~1550 kcal/day); meals were identical for both groups and for both the baseline and treatment weeks. Appropriate nutrition was determined according to the results of a Japanese health and nutrition survey,[15] and participants were instructed to finish the provided food. Other food or drink (particularly alcohol and caffeine) was not permitted, with the exception of water or caffeine-free, roasted barley tea.

The clinical research review board of the Kitasato Institute approved the study protocol (approval no. CRB3180002). This study was performed in accordance with the ethical standards laid down by the Declaration of Helsinki (as revised in Brazil 2013) and was registered on the Japan Registry of Clinical Trials (jRCT) website (Identifier: jRCTs031180035). Written informed consent was obtained from each study participant.

Participant selection. All participants were Japanese, aged ≥ 20 years, with no clinically significant medical history (e.g. liver dysfunction) and were not taking medications, supplements, or probiotics that could interfere with the study assessments or compromise participant safety.

Healthy subjects were defined as individuals who defecated almost every day for ≥ 6 months; subjects with ≤ 3 spontaneous bowel movements (SBMs) per week within 1 week of the run-in period were excluded.

Patients with chronic constipation were defined as those who satisfied the Rome IV criteria for functional constipation and had a mean Bristol stool form scale (BSFS) score of ≤ 3 during Week 2 of the run-in period. Exclusion criteria conformed with those of a previous study[11] and additionally included rescue medication use within 72 h after defecation and/or four times during the 2-week admission period or during the 1-week baseline.

Sample collection and analytical methods. Blood samples were collected before elobixibat administration on Days 1, 7, and (in patients with chronic constipation only) 14 for measurement of fasting C4, fibroblast growth factor 19 (FGF19; which downregulates BA synthesis), and BAs. A second sample for measurement of serum BAs was collected at 2, 4, 8, and 12 h after breakfast.

Fecal sampling was performed as collections every 48 h for 6 days during the baseline (Days 2–3, 4–5, and 6–7) and treatment (Days 9–10, 11–12, and 13–14) periods, with fecal weight measured immediately after each defecation. A stool sample was collected (~3 g) from the middle part of the feces and stored at −20°C for a single-stool measurement. The remaining stool was homogenized, and one-tenth of the total fecal weight was stored at −20°C. These 48-h fecal samples were subsequently thawed, mixed, and homogenized for measurement of BAs and stool liquidity.

Methods of BA extraction, analysis, and validation are described in Data S1. Serum BAs and biomarkers were measured at LSI Medience Corporation (Tokyo, Japan). Serum total BA was measured by enzymatic colorimetric assay and a BioMajesty™ instrument (series JCA-BM8060; JEOL Ltd., Tokyo, Japan). Serum concentration of FGF19 was measured using the Quantikine® enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN, USA). Fecal BAs were quantified at the Techno Suruga Laboratory (Shizuoka, Japan). For stool liquidity, a portion of the 48-h fecal samples was weighed and then dried. After measuring the dry weight obtained, the water content was calculated as a percentage.

Outcomes. The primary outcome was the change in total fecal BAs from baseline to the end of elobixibat treatment in patients with chronic constipation. Secondary outcomes included between-group differences in fecal and serum concentrations of total and individual BAs, and in serum C4 and FGF19 (both markers

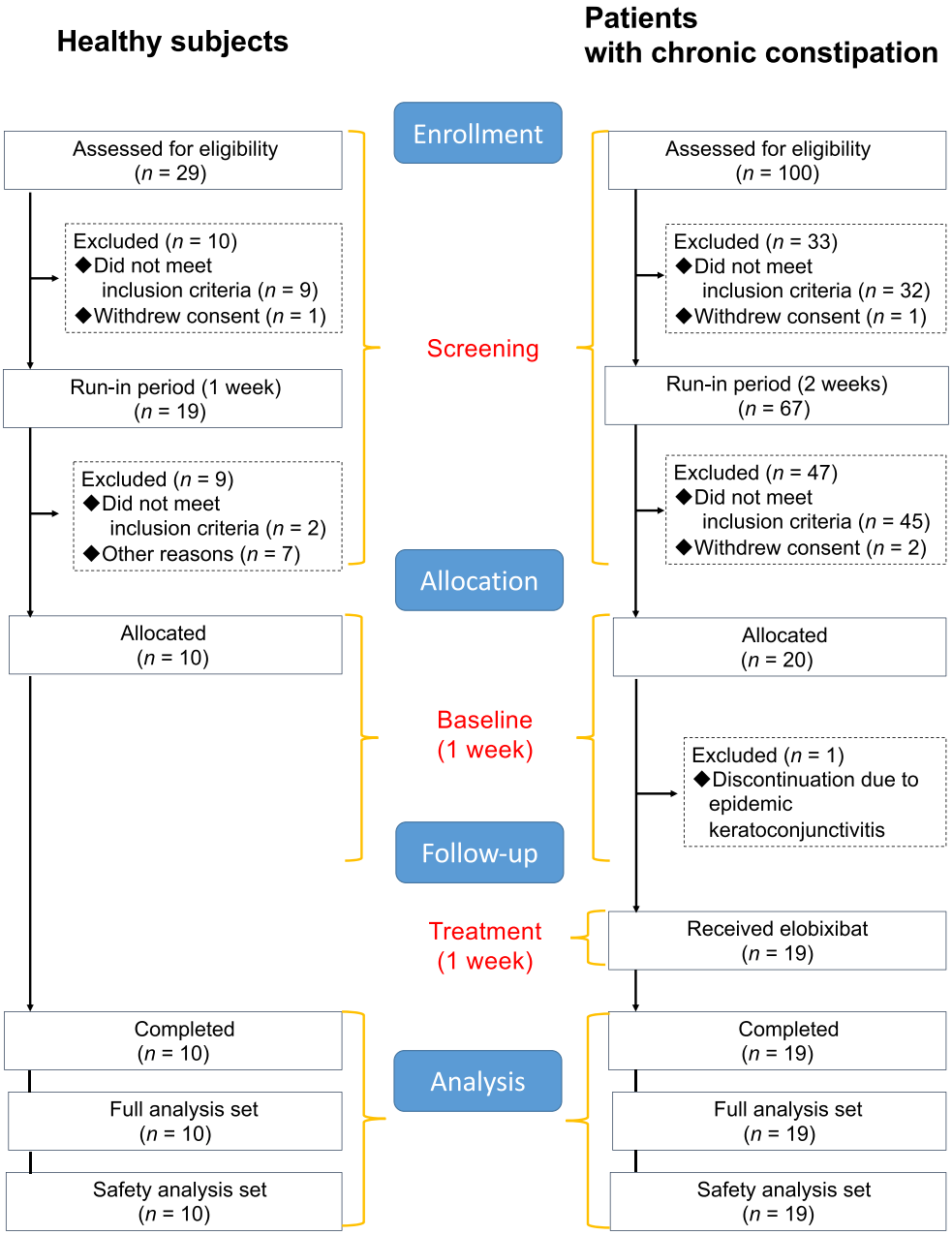

Figure 1 Study flow.

for BA absorption/malabsorption)[16]; changes in pharmacodynamic parameters after elobixibat administration in patients with chronic constipation; between-group differences in pharmacodynamic parameters; and effects of treatment on bowel characteristics. A post-hoc analysis explored the correlation of fecal total BA concentration between 3 g of single-stool samples collected over 6 days and a 6-day stool sample.

Bowel characteristics included fecal weight, stool liquidity, and stool consistency score assessed by the BSFS (averaged over the elobixibat treatment week), numbers of SBMs/week and complete SBMs (CSBMs)/week. Other constipation-related symptoms included straining, abdominal bloating, abdominal discomfort, and constipation severity; each was assessed using a 5-point subjective scale (none, mild, moderate, severe, and very severe).

Safety was assessed using adverse event (AE) monitoring and clinical laboratory tests; AEs were classified using the Medical Dictionary for Regulatory Activities version 21.1.

Statistical analyses. Baseline data between groups were compared with a Wilcoxon rank sum test. Baseline and post-treatment data from patients with chronic constipation

were compared using a Wilcoxon signed-rank test. A linear-trapezoidal method was employed to estimate the area under the effect curve (AUEC) for serum BAs. Total serum BAs were defined by the enzymatic assay value, whereas total fecal BAs were defined as the sum of individual BAs obtained by mass spectrometry. Fecal primary BAs were defined as CA + CDCA, and fecal secondary BAs as DCA + LCA. C4 and FGF19 concentrations falling below the lower limit of quantification (LLOQ) were imputed by half LLOQ concentration. Serum and fecal BA values falling below the LLOQ were imputed as zero. When a subject did not defecate within the 48-h collection period, the value was zero. Correlation of fecal BA concentrations between 3 g of single-stool samples collected over 6 days and 6-day stool samples was conducted using Lin's concordance correlation coefficient. Statistical data were analyzed using SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA) by IDD, Inc. (Tokyo, Japan).

Results

Participants. Overall, 10 healthy subjects and 20 patients with chronic constipation met the inclusion criteria, provided consent, and were admitted to the study center (Fig. 1). One patient with chronic constipation withdrew during the baseline period, resulting in 10 healthy subjects and 19 patients with chronic constipation. Of the 19 patients receiving elobixibat, 9 were down-titrated to 5 mg/day and 10 maintained a 10-mg dose. There were no protocol deviations.

Most participants were female (healthy subjects, 9/10 [90.0%]; patients with chronic constipation, 17/20 [89.5%]). Baseline demographics were generally comparable between the healthy subject and patient groups (mean ± standard deviation [SD] age: 42.8 ± 15.3 and 43.4 ± 12.4 years, respectively; body mass index: 20.5 ± 1.4 and 22.9 ± 3.0 kg/m^2, respectively). Table 1 shows bowel characteristics at baseline.

Fecal and serum bile acid characteristics at baseline. Patients with chronic constipation had significantly lower mean total fecal weight, stool liquidity, total fecal BAs, and primary BAs/gram of dry stool *versus* healthy subjects (Table 2). DCA concentrations were slightly lower and LCA 1.5-fold higher in patients *versus* healthy subjects.

The mean ± SD AUECs from 0 to 12.5 h ($AUEC_{0-12.5\ h}$) of total serum BAs at baseline in healthy subjects and patients with chronic constipation were 58.2 ± 21.2 and 80.1 ± 42.4 μmol·h/L, respectively. The mean ± SD changes in serum C4 and FGF19 concentrations from Days 1 to 7 were comparable between healthy subjects and patients with chronic constipation (C4: 24.2 ± 23.6 and 25.7 ± 16.3 ng/mL, respectively; FGF19: 199.3 ± 74.5 and

Table 1 Bowel function at baseline and effect of elobixibat treatment

Mean ± SD	Healthy subjects (*n* = 10)	Patients with chronic constipation (*n* = 19)	
	Baseline period	Baseline period	Elobixibat treatment period
SBMs per week	10.9 ± 6.0	2.0 ± 0.7	12.7 ± 2.8***
CSBMs per week	9.3 ± 6.6	0.5 ± 0.5	6.4 ± 4.0***
Stool consistency score	3.8 ± 1.0	2.1 ± 0.9	4.6 ± 1.1***

Data from the 7-day baseline period are shown for healthy subjects, and data from the 7-day baseline period and the 7-day elobixibat treatment period are shown for patients with chronic constipation.
***$P < 0.001$ *versus* patients with chronic constipation at baseline.
CSBM, complete spontaneous bowel movement; SBM, spontaneous bowel movement, SD, standard deviation.

Table 2 Fecal characteristics of participants at baseline

Mean ± SD	Healthy subjects (*n* = 10)	Patients with chronic constipation (*n* = 19)	*P* value†
Total wet fecal weight (g/day)	124.7 ± 52.9	34.0 ± 24.8	< 0.0001
Total dry fecal weight (g/day)	23.2 ± 4.7	9.5 ± 6.2	< 0.0001
Stool liquidity (%)	79.8 ± 4.7	69.5 ± 5.8	< 0.0001
Fecal total BAs (μmol/day)	688.2 ± 645.4	202.0 ± 208.3	0.0017
Total BAs per gram of wet stool (μmol/g)	5.1 ± 3.1	5.5 ± 3.0	0.5726
Total BAs per gram of dry stool (μmol/g)	27.6 ± 20.8	18.5 ± 10.0	0.4026
CA (μmol/g)	7.8 ± 13.8	0.9 ± 1.7	0.0306
CDCA (μmol/g)	5.0 ± 8.5	0.8 ± 1.1	0.0321
DCA (μmol/g)	9.1 ± 6.6	8.8 ± 5.8	0.9461
LCA (μmol/g)	4.6 ± 3.6	7.0 ± 3.9	0.1378
UDCA (μmol/g)	0.9 ± 1.2	0.5 ± 1.0	0.2249

Data from the 7-day baseline period are shown for healthy subjects and for patients with chronic constipation.
†Compared with healthy subjects at baseline.
BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; SD, standard deviation; UDCA, ursodeoxycholic acid.

185.7 ± 103.9 pg/mL, respectively), indicating that no patients had BA synthesis deficiency.

Effect of elobixibat on bile acids in patients with chronic constipation. Following elobixibat treatment, total fecal weight, total fecal BAs, and stool liquidity all increased significantly compared with baseline (Table 3). The total fecal BA excretion in elobixibat-treated patients was 2.1-fold higher than in healthy subjects. However, total dry fecal weights were comparable between groups.

In elobixibat-treated patients, significant changes in the primary BA concentration (25.4-fold increase from baseline), LCA concentration (3.9-fold decrease from baseline), and ursodeoxycholic acid concentration (3.8-fold increase from baseline) were observed; the DCA concentration remained almost unchanged (Table 3).

Following treatment, fasting serum C4 levels increased by 223%, and FGF19 levels decreased by 35%, indicating increased BA synthesis (Table 4). The $AUEC_{0–12.5\ h}$ of total serum BAs decreased (Table 4 and Fig. 2), while that of secondary BAs significantly decreased (Table 4 and Fig. S1). The $AUEC_{0–12.5\ h}$ of total serum BAs was slightly higher in elobixibat-treated patients *versus* healthy subjects.

There were no differences in total quantity of fecal BAs, total serum BAs, C4, or FGF19 concentrations in patients in whom elobixibat was down-titrated to 5 mg/day compared with those continuing to receive 10 mg/day.

Table 3 Effect of elobixibat on feces and fecal BAs in patients with chronic constipation

Mean ± SD	Patients with chronic constipation (n = 19)		P value[†]
	Baseline period	Elobixibat treatment period	
Total wet fecal weight (g/day)	34.0 ± 24.8	187.6 ± 91.9	< 0.0001
Total dry fecal weight (g/day)	9.5 ± 6.2	23.5 ± 8.1	< 0.0001
Stool liquidity (%)	69.5 ± 5.8	85.3 ± 6.4	< 0.0001
Fecal total BAs (μmol/day)	202.0 ± 208.3	1436.3 ± 808.5	< 0.0001
Total BAs per gram of wet stool (μmol/g)	5.5 ± 3.0	7.7 ± 1.8	< 0.01
Total BAs per gram of dry stool (μmol/g)	18.5 ± 10.0	58.2 ± 22.3	< 0.0001
CA (μmol/g)	0.9 ± 1.7	25.9 ± 14.7	< 0.0001
CDCA (μmol/g)	0.8 ± 1.1	17.3 ± 9.9	< 0.0001
DCA (μmol/g)	8.8 ± 5.8	8.9 ± 7.3	0.5412
LCA (μmol/g)	7.0 ± 3.9	1.8 ± 3.5	0.0002
UDCA (μmol/g)	0.5 ± 1.0	1.9 ± 2.7	0.0003

Data from the 7-day elobixibat treatment period are shown for patients with chronic constipation.
[†]Compared with patients with chronic constipation at baseline.
BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; SD, standard deviation; UDCA, ursodeoxycholic acid.

Table 4 Serum biomarker and bile acid measurements

Mean ± SD	Patients with chronic constipation (n = 19)		P value[†]
	Baseline period	Elobixibat treatment period	
Fasting C4 (ng/mL)	25.7 ± 16.3	83.1 ± 64.8	< 0.0001
Fasting FGF19 (pg/mL)	185.7 ± 103.9	119.9 ± 63.6	0.0124
Fasting total BAs (μmol/L)	3.8 ± 2.1	3.1 ± 2.0	0.1134
Total BAs $AUEC_{0–12.5\ h}$ (μmol/L*h)	80.1 ± 42.4	62.2 ± 22.8	0.0323
CA group (μmol/L*h)	7.3 ± 8.0	9.2 ± 11.3	0.8288
CDCA group (μmol/L*h)	36.2 ± 25.1	41.9 ± 17.2	0.0663
DCA group (μmol/L*h)	26.3 ± 22.0	5.2 ± 7.1	0.0002
LCA group (μmol/L*h)	0.8 ± 0.7	0.1 ± 0.2	< 0.0001
UDCA group (μmol/L*h)	9.6 ± 10.4	5.8 ± 5.4	0.0160

Data from day 14 (elobixibat treatment period) are shown for patients with chronic constipation. Individual BA groups indicate the total of tauroconjugated and glycoconjugated and unconjugated BAs.
[†]Compared with patients with chronic constipation at baseline.
$AUEC_{0–12.5\ h}$, area under the effect curve from 0 to 12.5 h; BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; SD, standard deviation; UDCA, ursodeoxycholic acid.

Correlation between stool samples. The correlation of total fecal BA concentration between 3 g of single-stool samples collected over 6 days and 6-day stool samples was assessed using a wet fecal weight. One patient with chronic constipation who defecated only once in 6 days had a total fecal weight of 9 g; therefore, 1 g was collected for the 6-day stool sample. There were no other patients whose 6-day single-stool sample was < 3 g. Correlation coefficients for healthy subjects, patients with chronic constipation at baseline, and elobixibat-treated patients were 0.8887, 0.8278, and 0.5979, respectively (Fig. 3).

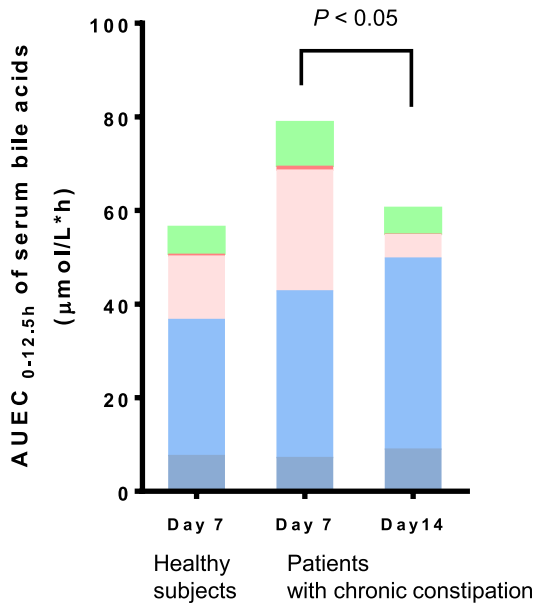

Figure 2 Mean serum concentration of bile acid groups. Each group indicates the total of tauroconjugated and glycoconjugated and unconjugated bile acids. Data on Day 7 (baseline period) are shown for healthy subjects, and data on Days 7 (baseline period) and 14 (elobixibat treatment period) are shown for patients with chronic constipation.$AUEC_{0–12.5\ h}$, area under the effect curve from 0 to 12.5 h; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid. , UDCA group; , LCA group; , DCA group; , CDCA group; , CA group.

Effects on bowel functions. Following elobixibat administration, SBMs and CSBMs in patients with chronic constipation were almost comparable with healthy subjects (Table 1). The BSFS stool consistency score also improved after treatment, from 2.1 (hard and lumpy) to 4.6 (soft and smooth). Additionally, subjective constipation-related symptoms improved with elobixibat (Fig. S2).

Safety. No subject experienced AEs or serious AEs leading to study discontinuation. In total, 8/10 (80%) healthy subjects experienced AEs, of which diarrhea (4/10 [40%]) and hard stool (4/10 [40%]) were the most frequent (Table 5). No AEs occurred in patients with chronic constipation during the baseline period; during the treatment period, 18/19 (94.7%) patients experienced AEs, of which abdominal pain (15/19 [78.9%]) and diarrhea (7/19 [36.8%]) were most frequently reported.

Discussion

This study confirmed that patients with chronic constipation have reduced excretion of feces and total fecal BAs, and decreased SBMs, compared with healthy subjects. After 1 week of elobixibat treatment, BA levels and constipation symptoms improved to levels comparable with healthy subjects. Both groups had similar changes in fasting serum C4 and FGF19 during the baseline period, indicating that no patients with chronic constipation had reduced BA synthesis at baseline according to the previously reported lower limit of normal (C4 < 5.05 ng/mL).[8] The excreted total BAs per gram of wet stool were comparable between groups,

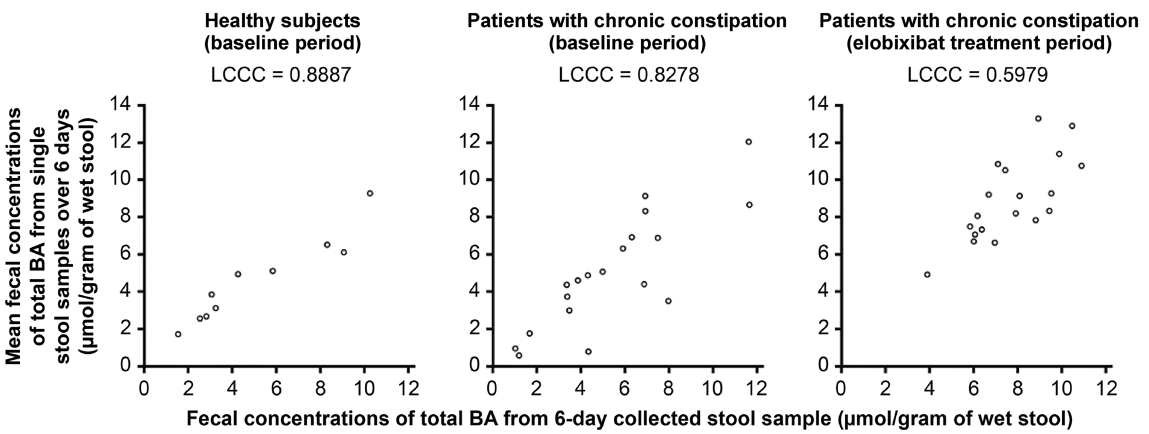

Figure 3 Correlation of total fecal BAs in wet stools. Scatter plots indicate the fecal concentration of total BAs per gram of wet stool from 3 g of stool samples collected over 6 days and from a 6-day collected stool sample. The correlation was assessed by LCCC.BA, bile acid; LCCC, Lin's concordance correlation coefficient.

Table 5 Summary of adverse events

AEs, *n* (%)	Healthy subjects† (*n* = 10)	Patients with chronic constipation‡ (*n* = 19)
Any AE	8 (80.0)	18 (94.7)
AE leading to discontinuation	0	0
Abdominal pain	1 (10.0)	15 (78.9)
Diarrhea	4 (40.0)	7 (36.8)
Hard stool	4 (40.0)	0
Abdominal distension	0	2 (10.5)
Nausea	0	2 (10.5)
Upper abdominal pain	0	2 (10.5)
Increased blood triglycerides	2 (20.0)	0
Back pain	0	1 (5.3)
Chills	0	1 (5.3)
Erythema	0	1 (5.3)
Headache	0	1 (5.3)
Increased alanine aminotransferase	0	1 (5.3)
Increased transaminase	0	1 (5.3)
Thirst	0	1 (5.3)
Eyelid swelling	1 (10.0)	0

†During the baseline period.
‡During the elobixibat administration period (no AEs were observed in this group during the baseline period).
AE, adverse event.

although the excreted total BAs per gram of dry stool were lower in patients with chronic constipation.

Notably, the $AUEC_{0–12.5\ h}$ of total serum BAs (particularly secondary BAs) was higher in patients with chronic constipation *versus* healthy subjects. Given that patients had decreased fecal BA excretion and lower SBMs despite their normal BA synthesis rates, the increase in total serum and secondary BAs is likely due to stool retention, resulting in passive BA reabsorption further down the colon. Stool retention also provides more time and opportunity for the colonic dehydroxylation of CDCA to LCA and may cause the increased concentration of fecal LCA in patients with chronic constipation. These data are consistent with a previous study that reported that fecal LCA could be a predictor for low fecal weight, stool frequency, and consistency score.[17] Unexpectedly, the concentration of DCA, another secondary BA, was slightly lower in patients with chronic constipation, even after elobixibat treatment. We speculate that following elobixibat administration, stool-holding time in the large intestine shortens, and passive absorption and LCA levels decrease. However, because of the balance between BA production and absorption, DCA levels would remain unchanged. Further investigation is needed to confirm this. DCA is a secretory BA that promotes colonic secretion,[18] thereby increasing primary BAs while maintaining its own level. This mechanism might be one of the essential events leading to the improvement of constipation symptoms after elobixibat treatment.

Elobixibat treatment was associated with a 7-fold higher mean fecal BA excretion, with an almost 10 SBM/week increase from baseline in patients with chronic constipation. After elobixibat administration, concentrations of individual fecal BAs, except for DCA, showed significant change. The percentage of fecal primary BAs (CA + CDCA) was considerably higher (74.1%) after *versus* before treatment (9.1%), consistent with a previous study that administered an IBAT inhibitor to healthy subjects.[19] Conversely, a recent prospective study reported a smaller (1.5-fold) increase in fecal BAs after 2 weeks of treatment with elobixibat in patients with chronic constipation.[20] The reasons for this discrepancy may include the study design (random sampling of fecal samples and no control of dietary nutrition), patient demographics (older age *vs* our study), and frequency of bowel movements during treatment (low *vs* our study).[20]

In serum BA concentrations assessed with $AUEC_{0–12.5\ h}$, primary BAs were increased; this was attributed to increased hepatic BA synthesis and high levels of primary BAs in the colon, resulting in increased passive colonic absorption. Serum secondary BA levels were considerably lower in elobixibat-treated patients than in healthy subjects. Thus, primary and secondary BA levels in the colon following elobixibat treatment were comparable, and SBM frequency increased, leading to shorter colonic retention time and a reduction in BA biotransformation by colonic microbiota.

Abdominal pain and diarrhea were the most frequent AEs, consistent with the results of a large-scale, long-term phase 3 trial conducted in Japan.[11] High AE frequency was also observed in the previous phase 1 trial;[21] however, AE frequency decreased during long-term elobixibat administration.[11] Notably, the healthy subjects in this analysis also experienced fecal-related AEs (diarrhea and hard stools). The hospitalization period required for the study baseline may have caused stress for healthy individuals, impacting bowel function. High fecal total BAs per gram of wet stool have previously been associated with diarrhea in patients with IBS[22]; in the current study, fecal total BAs per gram of wet stool in elobixibat-treated patients were 1.5-fold higher than in healthy subjects. Increased fecal BAs are associated with increased wet fecal weight, and stool liquidity is most likely caused by the increase in both BA synthesis and excretion after elobixibat administration.

The evaluation of sample collection procedures found that concentrations of total BAs per gram of wet stool had moderate correlations during the baseline period in both groups. However, the correlation coefficient in patients with chronic constipation during the treatment period was low, indicating that the BA and water content in a single stool of elobixibat-treated patients were heterogeneous.

Limitations of this study include the small sample size and lack of a placebo or active control group. Although the impact of elobixibat treatment on colonic transit has been previously established,[23,24] whether microbial composition in the intestine is altered following elobixibat treatment warrants further investigation. Another limitation was the 1-week study duration, which meant we could not determine the long-term impact of elobixibat administration on BAs, though long-term efficacy of elobixibat has been confirmed; we speculate that BA levels in stools would increase, although further investigation is needed to confirm this.

In conclusion, this inpatient study was able to minimize factors such as food intake that affect BA synthesis rates. Improvement of constipation after elobixibat treatment is associated with increased total BAs and, in particular, primary BAs (Fig. S3). Further long-term studies with larger sample sizes are needed to clarify microbial compositions associated with increased BA synthesis after elobixibat administration.

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