

The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid

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SUMMARY

Background

Bile acid diarrhoea is a common cause of chronic diarrhoea, occurring as a primary condition or secondary to ileal disease or resection. Many patients have reduced levels of the ileal hormone fibroblast growth factor 19 (FGF19), an inhibitory regulator of hepatic bile acid synthesis, secreted in response to farnesoid X receptor (FXR) activation.

Aim

To investigate whether obeticholic acid, a potent FXR agonist, could increase FGF19 in patients with bile acid diarrhoea, and produce clinical benefits.

Methods

After a 2 week run-in when bile acid sequestrants were discontinued, patients with previously diagnosed primary bile acid diarrhoea ($n = 10$), secondary bile acid diarrhoea ($n = 10$) or idiopathic chronic diarrhoea ($n = 8$), received oral obeticholic acid 25 mg daily for 2 weeks. Serum FGF19, total bile acids and 7α -OH-4-cholesten-3-one (C4) were measured, symptoms recorded and a diarrhoea index calculated.

Results

In primary bile acid diarrhoea, obeticholic acid increased median fasting FGF19 (133–237 pg/mL, $P = 0.007$) and significantly reduced fasting C4 and bile acid responses. Improvements occurred in median stool frequency (–24% after 2 weeks treatment, $P = 0.03$), stool form (–14%, $P = 0.05$) and diarrhoea index (–34%, $P = 0.005$). In the secondary bile acid diarrhoea group, significant clinical improvements were found predominantly in patients with shorter ileal resections. Symptoms of abdominal pain and urgency improved. FGF19 and bile acids changed in the control group, without significant clinical improvement. Total and LDL-cholesterol increased and triglycerides decreased. Obeticholic acid treatment was well tolerated.

Conclusions

This proof-of-concept study indicates that obeticholic acid stimulates FGF19, reduces bile acid synthesis and produces clinical benefits in bile acid diarrhoea. FXR agonists have therapeutic potential in chronic diarrhoea. EudraCT 2011-003777-28; Clinical Trials: NCT01585025

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INTRODUCTION

Bile acid diarrhoea (BAD) occurs when excess amounts of bile acids (BA) enter the colon and induce secretion and motility changes. The majority of conjugated BAs are absorbed in the terminal ileum and undergo enterohepatic circulation; only a small portion of BAs are not reabsorbed and pass into the colon. BAD was first recognised in ileal disease or after ileal resection, most commonly due to Crohn's disease.¹ This form of BAD is thought to be secondary to BA malabsorption. BA sequestrants have been used as treatment for many years.²

Primary BAD is the condition in which chronic diarrhoea is associated with excessive faecal BA loss, occurring in the absence of overt ileal disease.³ This condition is often unrecognised and undiagnosed,⁴ as specific tests are not widely available. Diagnostic methods have recently been reviewed.⁵ Quantifying faecal BA is the gold standard, but this test is unpleasant and unpopular. Blood levels of the BA precursor 7 α -hydroxy-4-cholesten-3-one (C4) can be used to detect increased BA synthesis. Measurement of retention and loss of a radiolabeled synthetic BA, ⁷⁵Se-homocholic acid taurine (SeHCAT), has been used in many countries (but not the USA) since 1981⁶ and considerable experience has been accumulated in multiple small and larger studies.^{7–9} In 2009, a systematic review⁷ showed that about 30% of patients who otherwise would be diagnosed with diarrhoea-predominant irritable bowel syndrome (D-IBS) have reduced BA retention of SeHCAT less than 10% at 7 days, consistent with primary BAD. This review estimated the population prevalence to be around 1% of all adults.

However, since most patients with primary BAD do not have impaired BA absorption, an alternative mechanism of BA overproduction has been suggested.^{10, 11} Hepatic BA synthesis is regulated through feedback inhibition by a hormone produced in the ileum, fibroblast growth factor 19 (FGF19).¹² Reduced median serum levels of FGF19 were shown in BAD, associated with raised C4 levels, indicating increased BA synthesis.¹⁰ These findings were confirmed in a large series of chronic diarrhoea patients who all had C4 measurements¹³ and also in a prospective series of patients who had SeHCAT tests.¹⁴ Further support for the role of an impaired FGF19 system in chronic diarrhoea has come from a number of animal studies; these studies and other factors that could influence FGF19 were recently reviewed.⁸

FGF19 production in the ileum is stimulated by BA binding to the farnesoid X receptor (FXR) and activating transcription.¹² Using explants of human ileum, we

showed that FGF19 transcripts and protein levels were potently stimulated by chenodeoxycholic acid (CDCA), the most active natural FXR agonist.¹⁵ More potent FXR agonists have been synthesised with the aim of treating liver and other disorders.¹⁶ Obeticholic acid (OCA, also known as 6 α -ethyl-CDCA and INT-747) is about 100-fold more potent an FXR agonist than CDCA.¹⁷ Clinical trials of OCA in primary biliary cirrhosis (PBC) and nonalcoholic steatohepatitis (NASH) have been published or are in progress, with treated patients showing apparent dose-dependent increases in FGF19.^{18–21}

We hypothesised that **if potent FXR agonists such as OCA were able to stimulate FGF19 in patients with BAD, this would reverse the impaired regulation of BA synthesis and, by reducing BA production, would lead to clinical improvement.** We anticipated that the effect would be most marked in primary BAD, but that impaired BA absorption could limit the effect in secondary BAD. To test this hypothesis, we have performed a proof-of-concept study in three groups of patients with primary BAD (SeHCAT <15%), secondary BAD and controls with idiopathic chronic diarrhoea (SeHCAT >15%).

METHODS

Study design

This was an open-label, single-centre pilot study of mechanisms, safety and symptom response of obeticholic acid treatment in patients with BAD. It was an investigator-initiated trial, known as OBADIAH1, sponsored by Imperial College London and Imperial College Healthcare NHS Trust and registered with EudraCT (2011-003777-28) and ClinicalTrials.gov (NCT01585025). The drug was provided by Intercept Pharmaceuticals, Inc. (San Diego, CA, USA). Ethical approval was obtained from National Research Ethics Service Committee London Brent (REC reference 12/LO/0123). Use of OCA was approved by the Medicines and Healthcare Regulatory authority (MHRA, London, UK). Written informed consent was obtained from all subjects who were fully briefed that they were free to withdraw at any point in the study. The study was conducted according to Good Clinical Practice guidelines. An independent Data and Safety Monitoring Committee reviewed study progress and safety data. All authors had access to the study data and approved the final manuscript.

The design of the trial is shown in Figure 1. This lasted 6 weeks, consisting of a 2-week run-in period (weeks 1 and 2), 2 weeks of treatment with oral OCA

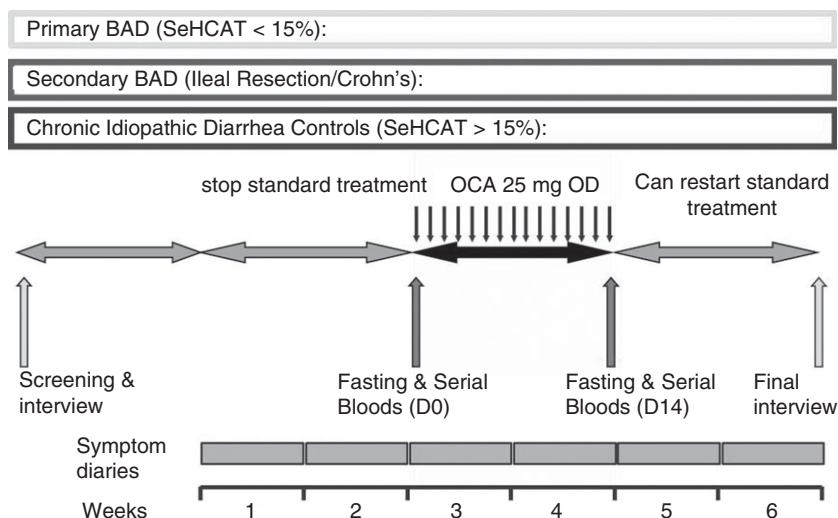


Figure 1 | Design of the OBADIAH1 study. Three groups of patients were studied over 6 weeks. Obeticholic acid (OCA) was given in weeks 3 and 4 with fasting and serial blood sampling at the beginning and end of this period on day 0 (D0) and day 14 (D14).

25 mg daily (weeks 3 and 4) and a 2 week follow-up period off OCA (weeks 5 and 6). Treatment with bile acid sequestrants was discontinued at the start of the trial; loperamide (up to 16 mg/day) was allowed as rescue therapy. Daily symptom diaries were used to record stool frequency, stool form (type) using the seven-point Bristol Stool Form Scale (BSFS),²² loperamide usage, abdominal pain, urgency and bloating scores on a ten-point scale, ranging from none to moderate to severe. The number of hours of pain experienced was also recorded. Patients were asked to record any adverse events in their symptom diaries. Weeks 5 and 6 diaries were not returned by several patients.

Subjects attended on the first and last days of the 2 week treatment period (D0 and D14), having been asked to fast from 9 pm the night before. Blood was taken in the fasting state and then OCA was given on those days. Subjects were then given a standard mixed content breakfast. Further serial postprandial/OCA blood samples were taken at one-hourly intervals for six hours. A standard mixed content lunch was consumed after three hours and sampling continued for three hours after this.

Subjects

Thirty-five patients with chronic diarrhoea were initially recruited from gastrointestinal clinics at the hospitals of Imperial College Healthcare NHS Trust. They were aged 24–74 years and all had described symptoms of passing an average of more than three soft or watery stools per day for at least the past 3 months. Although some patients experienced episodes of abdominal pain, the Rome inclusion criteria for IBS were not formally investigated but most patients would be classed as IBS-D or chronic painless diarrhoea. All patients had received

standard investigation for diarrhoeal symptoms prior to enrolment; other causes of diarrhoea such as colorectal neoplasia, ulcerative colitis, coeliac disease, chronic pancreatitis, drug-induced diarrhoea, active infection or lactose intolerance had been excluded. The patients in the secondary BAD group had Crohn's disease and/or ileal resection. Patients with other clinically significant disorders, including liver disease, were excluded.

The SeHCAT test was used for diagnosis of BAD. Patients with primary BAD all had SeHCAT 7 day retention $\leq 10\%$. In the secondary BAD group SeHCAT was not necessary, as previous studies have shown that over 90% of patients with Crohn's disease, ileal resection and chronic diarrhoea have a SeHCAT $\leq 10\%$.^{23, 24} Two secondary BAD patients with Crohn's disease did not have resections, but had SeHCAT tests with retention values of 10% and 4%, respectively. The median ileal resection length was 33.5 cm, range 0–71 cm. Several patients also had short colonic resections. Patients in the control group with idiopathic chronic diarrhoea all had normal SeHCAT 7 day retention of $>15\%$.

Sample analysis

Blood samples were separated, aliquoted and stored at $-80\text{ }^{\circ}\text{C}$. Assays were performed by operators who were unaware of the clinical condition of the patients. FGF19 was measured by ELISA sandwich assay (FGF19 Quantikine ELISA kit; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Serum C4 levels were determined by the previously documented procedure in the Clinical Chemistry Department, Western General Hospital, Edinburgh.²⁵ Following solid phase extraction, samples were analysed by high performance liquid chromatography. Total serum BA was measured by an enzy-

matic colorimetric method using 3 α -hydroxysteroid dehydrogenase. Liver function tests (LFTs) and lipids were measured using standard procedures at the core Clinical Chemistry Laboratory at Hammersmith Hospital.

Statistical analysis

The primary endpoints were the changes in fasting FGF19 in each of the three groups between the samples taken before the first dose of OCA (D0) and after 2 weeks (D14) before the last dose. Secondary endpoints included changes in fasting C4 and BA, liver enzymes, fasting lipids and clinical symptoms and comparisons between the three groups. Peak values during the serial sampling (usually at five or six hours) were identified and 0–6 h integrated response (area under curve; AUC) values for FGF19 and BA were compared on D0 and D14.

Symptoms recorded in the patient diaries were analysed as total weekly stool frequency and mean weekly BSFS. Total weekly loperamide use (in mg) was recorded. As patients can respond differently, to detect a global response involving changes in any of these parameters, we also prospectively developed a weekly diarrhoea index (stool index), calculated using the formula ([weekly stool frequency * mean BSFS] + loperamide use [weekly mg*3]). This factor of three equates one 2 mg dose of loperamide to one type 6 stool. Comparisons were made principally between symptom data for W2, the second week of the run-in period off specific treatment, with data for W4, the second week of OCA treatment to allow for drug levels to stabilise and effects to fully develop. As several patients dropped out before dosing with problems unrelated to medication effects, a modified intention-to-treat analysis was performed.

Data are usually reported as medians and interquartile ranges (IQR). Nonparametric tests were used to look for treatment effects including Kruskal–Wallis comparisons between the three groups, Mann–Whitney *U* unpaired or Wilcoxon paired rank tests and Spearman rank correlations. Statistical analyses were performed using Winstat for Excel (R. Fitch Software, Bad Krozingen, Germany). *P* values <0.05 were considered significant.

RESULTS

Study patients

A total of 35 patients entered the study, but seven patients (three in the secondary BAD group and four in the chronic idiopathic diarrhoea control group) withdrew before the first dose of OCA for personal reasons including lack of time, child's illness and bereavement.

None of these patients received OCA and is not included in the analyses. Consequently, 28 patients (10 primary BAD, 10 secondary BAD and 8 idiopathic diarrhoea controls) received OCA and had blood sampling. Further details of these groups are shown in Table 1.

FGF19

In the primary BAD group, at the end of the 2 week run-in period (D0), the baseline fasting FGF19 median value (133 pg/mL) was similar to that found before (Table 2).^{10, 14} At the end of week 4, after 2 weeks OCA treatment (D14), the median fasting FGF19 (237 pg/mL) was significantly higher (*P* = 0.007) and similar to that found in healthy controls previously.¹⁰ The median and individual changes in FGF19 are shown in Figure 2 (a, d). Nine of the ten patients with primary BAD had a positive increment with the median percentage increase over baseline being 71%, IQR 9–102%.

Comparisons with the secondary BAD and idiopathic diarrhoea control groups (Table 2) show that the three groups differed significantly in the baseline (D0) FGF19 values (*P* = 0.002, Kruskal–Wallis). The baseline (D0) median fasting FGF19 in the secondary BAD group (32 pg/mL) was significantly lower than in the primary BAD and idiopathic control groups (*P* = 0.001 and 0.004 respectively, Mann–Whitney test). The control group

Table 1 | Demographics of the three patient groups in this study

	Primary BAD	Secondary BAD	Idiopathic controls
Number	10	10	8
Sex (%)			
Male	3 (30)	3 (30)	5 (63)
Female	7 (70)	7 (70)	3 (37)
Age (years)			
Median	47	45	39
Range	24–74	27–71	25–68
SeHCAT (7 days retention %)			
Number	10	6	8
Median	4.8	0.6	25.7
Range	0.8–9.3	0–10.4	16.0–40.0
No ileal resection (<i>n</i>)	10	2	8
Previous ileal resection (<i>n</i>)	0	8	0
Median length (cm)	–	34	–
Range (cm)	–	15–71	–
15–45 cm (<i>n</i>)	–	5	–
>45 cm (<i>n</i>)	–	3	–
Previous BA sequestrant use (<i>n</i>)			
Yes	5	4	0
No	5	6	8

Table 2 | FGF19, C4 and Bile Acids: fasting, 6 h AUC and peak values on D0 and D14

	Primary BAD (n = 10)			Secondary BAD (n = 10)			Idiopathic controls (n = 8)			Overall (n = 28)		
	D0	D14	P	D0	D14	P	D0	D14	P	D0	D14	P
FGF19 (pg/mL)												
Fasting	133	237	0.007	32	46	0.11	116	194	0.12	74	152	0.0004
	102–168	116–302		24–42	24–72		57–186	126–344		31–133	49–250	
6 h response	3945	3825	0.72	401	457	0.51	1644	2099	0.13	1642	1972	0.13
AUC	2609–4834	2515–6129		267–1086	316–1016		1247–2776	1972–2341		484–3321	536–3011	
6 h peak	1278	1216	0.51	102	126	0.96	530	588	0.40	496	560	0.90
	764–1723	687–1928		47–296	58–229		392–805	480–800		127–1011	158–902	
C4 (µg/L)												
Fasting	16*	3*	0.03	104	56	0.11	9	1	0.02	20	4	0.001
	11–37	1–17		48–134	14–122		3–14	1–3		9–96	1–39	
Bile acids (µmol/L)												
Fasting	1.5	0.9	0.13	2.5	2.5	0.12	1.5	1.0	0.12	2.0	1.0	0.04
	1.0–4.0	0.9–3.0		2.0–6.0	1.0–4.0		1.0–2.8	0.9–1.8		1.0–3.8	0.9–3.0	
6 h response	34.5	20.9	0.02	32.0	22.0	0.04	29.0	17.0	0.02	31.5	20.0	0.0001
AUC	22.5–64.8	12.9–30.3		20.5–39.0	15.8–30.0		23.2–44.7	10.6–20.0		22.3–39.0	12.9–30.0	
6 h peak	7.5	4.0	0.02	6.5	4.5	0.21	8.0	4.0	0.02	7.0	4.0	0.0001
	4.0–15.0	2.8–7.5		3.8–9.3	3.0–6.3		7.0–11.5	3.0–4.0		4.5–10.8	3.0–6.0	

AUC, area under the curve.

Values are medians and IQR. D0 and D14 values were compared by Wilcoxon paired rank tests. Significant values ($P < 0.05$) are in bold.

* Data were incomplete in two patients.

fasting values were widely distributed resulting in a lower median in this small group compared to that seen in our larger series.¹⁴

The changes in FGF19 after OCA treatment were variable in the secondary BAD group (median percentage increase over baseline = 25%, IQR –6% to 140%; $P = 0.11$) with some patients having large increases and others having only small changes. In the idiopathic control group, FGF19 increases were also variable and did not reach significance (median percentage change = 130%, IQR –14% to 304%; $P = 0.12$).

As expected, FGF19 increased in the serial blood samples taken for 6 h after the OCA doses. The groups differed in D0 AUC (Kruskal–Wallis $P = 0.004$) and peak FGF19 values ($P = 0.0003$) with the responses being significantly highest in primary BAD, followed by the idiopathic controls and least in the secondary BAD groups. There were no significant differences between the responses on D0 compared to D14 in FGF19 AUC or 6 h peak in any of the groups (see Data S1).

7 α -hydroxy-4-cholesten-3-one (C4)

Fasting C4 values were significantly different between the groups (Table 2). Median values in patients with secondary BAD were much higher than those with primary BAD, which in turn were higher than chronic diarrhoea controls. OCA treatment reduced C4 in all groups, but this did not reach significance in the overall second-

ary BAD group. Figure 2 (b,e) show values in primary BAD.

Total bile acid values

Fasting BA values were not different between the groups (Table 2). After 2 weeks of OCA treatment median fasting values were significantly lower overall, but this did not reach significance in any group. The BA increases after OCA and meals during the 6 h serial sampling showed significant changes after 2 weeks of OCA treatment (Figure S1). The median BA AUC was significantly lower in all groups; the data in the primary BAD group are shown in Figure 2 (c,f). The BA peak was significantly lower in most patients.

Changes in clinical symptoms

The changes in clinical parameters that occurred during the 6 weeks of the study are shown for the primary BAD group in Figure 3. Stool type worsened slightly during the 2-week run-in period as other medication (apart from loperamide) was stopped. There were statistically significant reductions in stool frequency and BSFS stool type during the 2 weeks of OCA therapy; median values and significance after 2 weeks are shown in Table 3. Loperamide was used by two primary BAD patients, though both reduced their total dosage. The calculated stool index improved in every patient, with a median percentage change of –22% ($P = 0.01$) after 1 week of treatment

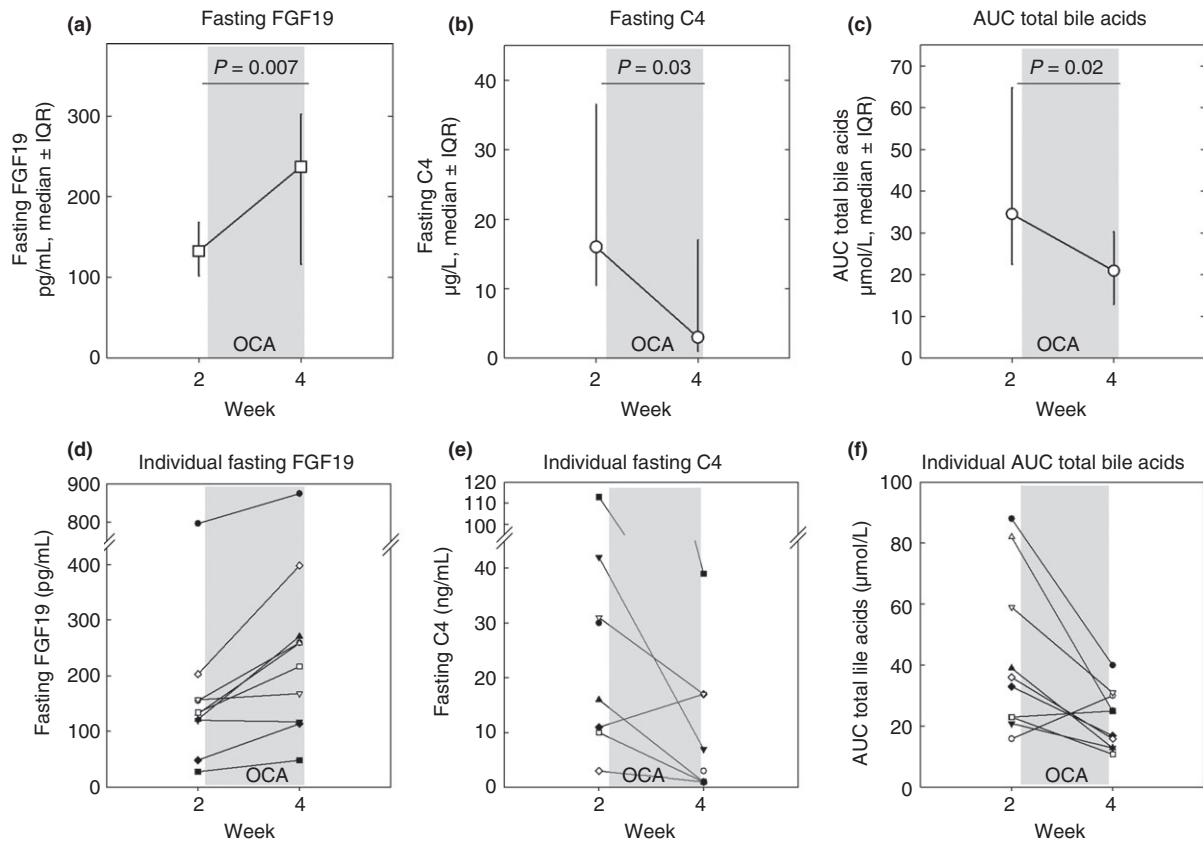


Figure 2 | Fasting serum FGF19, C4 and bile acid 6 h area under the curve (AUC) values in patients with primary BAD before and after treatment. OCA 25 mg/day was given between weeks 2 and 4 (DO and D14). Median values and Interquartile ranges (IQR) are shown in a–c. Individual patient values are shown in d–f.

between W2 and W3, and further improvement with a second week of treatment of -12% ($P = 0.07$) giving a total change with 2 weeks treatment of -34% ($P = 0.005$). The beneficial effects continued after stopping OCA therapy into week 5 but symptoms worsened in week 6, when two patients restarted BA sequestrants.

The effects on clinical symptoms in each of the three groups are shown in Table 3. In the secondary BAD group, there were significant changes with OCA therapy in stool form ($P = 0.04$) and in the stool index ($P = 0.03$). The median percentage index change was -29% , similar to that found in the primary BAD group. These secondary BAD patients' symptoms were variable, with some being more severely affected than the primary BAD patients. One patient had a weekly stool number of over 100 type 6–7 stools. Four patients needed loperamide rescue therapy and only one was able to decrease dosage while on OCA.

The patients with long resections responded less well than those with short or no resections and we aimed to define a length below which patients would respond.

Limiting the analysis to the seven patients with an ileal resection length <45 cm produced significant improvements in stool number ($P = 0.03$), stool form ($P = 0.02$) and index ($P = 0.02$). This group also experienced significant changes in FGF19 (38 to 50, $P = 0.03$) and C4 (90 to 32, $P = 0.02$).

Patients in the idiopathic chronic diarrhoea control group showed no significant changes in median stool number, form or index. Changes were generally small; the median percentage index change was $+6\%$.

Associated symptoms showed improvement in many patients. There was a significant median reduction in urgency score in the primary BAD group of -49% ($P = 0.03$) and in the secondary BAD patients with resection <45 cm of -56% ($P = 0.03$). Pain scores in hours (percentage change -64% , $P = 0.03$) and severity (percentage change -71% , $P = 0.04$) both improved in the secondary BAD group. Pain was not a problem in the primary group, where only five patients reported any episode of pain. These were mild and did not change significantly on treatment. None of these symptoms changed signifi-

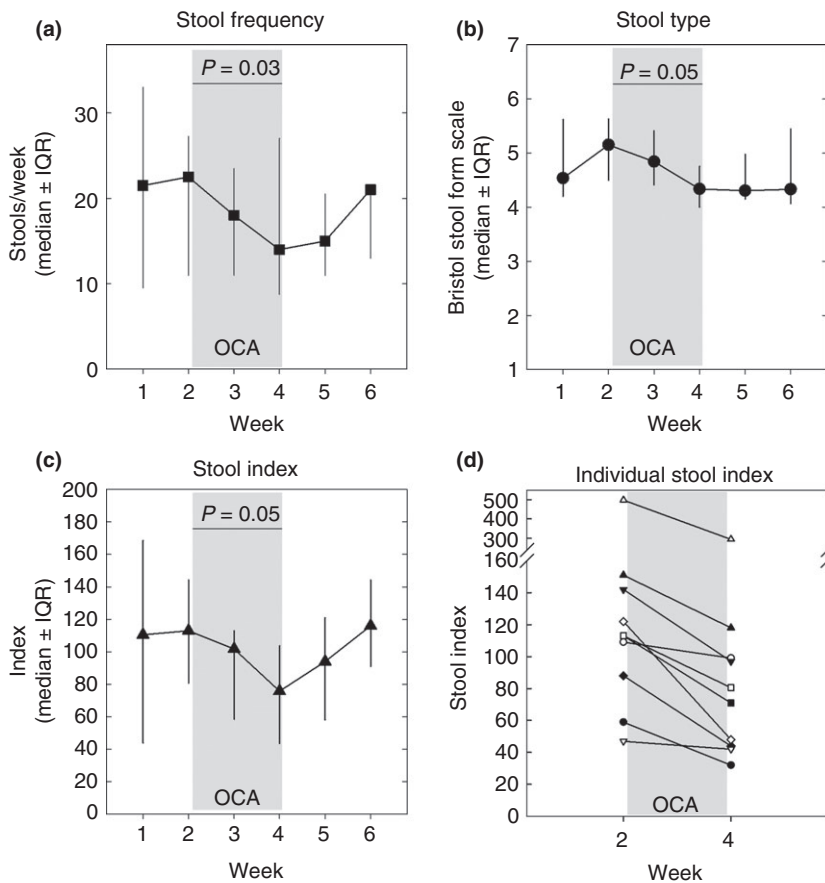


Figure 3 | Clinical symptoms in primary BAD patients. (a–c) show medians and IQR for all 6 weeks of the study. (a) weekly stool number (frequency); (b) stool type (Bristol stool form scale); (c) stool index (see text for calculation); (d) individual changes in stool index between weeks 2 and 4.

	Table 3 Changes in clinical symptoms with OCA treatment between weeks 2 and 4								
	Weekly number of stools			Mean stool form (BSFS)			Stool index*		
	W2	W4	P	W2	W4	P	W2	W4	P
Primary BAD									
n = 10	23	14	0.03	5.2	4.3	0.05	113	76	0.005
	11–27	9–27		4.5–5.6	4.0–4.8		81–144	44–104	
Secondary BAD									
n = 10	23	17	0.17	6.0	5.6	0.04	132	127	0.03
	14–44	17–42		5.3–6.9	4.6–6.7		72–473	47–321	
Resection <45 cm									
n = 7	17	12	0.03	5.7	5.2	0.02	95	62	0.02
	15–29	10–27		4.4–6.2	4.2–6.0		71–202	42–166	
Idiopathic									
n = 7†	15	18	0.31	4.9	4.9	0.74	96	83	0.61
	11–17	13–19		4.3–5.9	4.1–5.0		69–100	65–101	
Overall									
n = 27	17	17	0.12	5.4	4.9	0.01	100	83	0.0007
	11–28	11–27		4.5–6.0	4.1–5.3		72–151	49–118	

Values are medians and IQR. Week 2 (W2) and week 4 (W4) values were compared by Wilcoxon paired rank tests. BSFS = Bristol Stool Form Scale. Significant values ($P < 0.05$) are in bold.

* The stool index = ([weekly stool frequency × mean BSFS] + loperamide use [weekly mg × 3]).

† One patient did not return diaries that could be analysed.

cantly in the idiopathic diarrhoea control group, where all had some episodes of pain. Overall, significant improvements in urgency (median percentage change -29% , $P = 0.01$), pain hours and severity (both $P = 0.04$) were confirmed. Bloating scores were unaffected.

Relationships of change in clinical symptoms to FGF19 and bile acids

In the primary BAD group, the percentage change in stool number was associated with the percentage change in fasting FGF19 and BA AUC (Figure 4). This BA response was inversely correlated with the change in fasting FGF19. In addition, the percentage change in fasting C4 was significantly associated with reduced urgency score ($r = 0.65$, $P = 0.04$) and with the change in FGF19 6-h response ($r = -0.62$, $P = 0.05$), indicating that increases in FGF19 resulted in reduction in C4 and new BA synthesis.

In the secondary BAD group, the change in stool number was associated with the percentage change in fasting FGF19 ($r = -0.56$, $P = 0.05$). The resection length was associated overall with percentage changes in stool form ($r = 0.54$, $P = 0.05$), stool number ($r = 0.46$, $P = 0.09$) and in urgency ($r = 0.77$, $P = 0.01$). Resection length was also significantly associated with FGF19, BA and C4 values; these and additional relationships are described in Data S2.

Tolerability, liver enzymes and lipid analyses

Obeticholic acid was well tolerated and only a few minor adverse effects were reported of limited clinical significance. Three patients (two in the secondary BAD group and one in the idiopathic group) reported mild head-

aches during OCA treatment in week 3 or 4. In the absence of a placebo-treated group, it is uncertain whether these were possibly related to OCA treatment. Another patient with Crohn's disease experienced worsening of abdominal pain after starting OCA and did not have a bowel movement for 24 h. Her symptoms settled on omitting OCA for 1 day and then continuing with OCA 25 mg on alternate days.

Lipid measurements showed higher values for fasting total and LDL-cholesterol (median increases of 10.4% and 19.7% , respectively, both $P < 0.001$ overall) and a reduction in triglycerides (median decrease of -11.8% , $P = 0.06$) after 2 weeks OCA treatment. HDL-cholesterol did not change significantly. Similar magnitudes of changes were found in all three groups (Table S1) and are comparable to those published previously.²⁰ There were no significant changes in median values of liver biochemistry parameters, including alkaline phosphatase, alanine transaminase, bilirubin and albumin, which all remained in the normal range.

DISCUSSION

This is the first study testing a potent FXR agonist, OCA, as a novel treatment for patients with chronic diarrhoea. Two weeks of therapy with OCA produced statistically significant increases in FGF19 in the primary BAD and secondary BAD group (patients with ileal resection length <45 cm), which were in turn associated with reduced BA synthesis and clinical improvements. These findings are consistent with predictions from our previous work.

Increased FGF19 levels, together with reduced C4 and BA levels, clearly show FXR activation by OCA in these

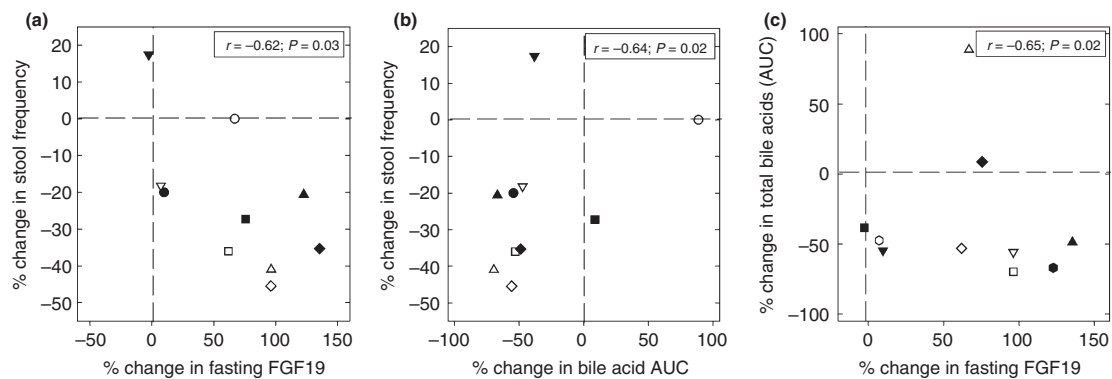


Figure 4 | Relationships of percentage change in stool frequency, fasting FGF19 and bile acid AUC in primary BAD patients after 2 weeks OCA treatment. The various open and closed symbols indicate individual patients. (a) changes in stool number and fasting FGF19; (b) changes in stool number and bile acid AUC; (c) change in bile acid AUC and fasting FGF19. Spearman correlation coefficients and P values are shown.

patients with chronic diarrhoea. FGF19 values vary greatly, in keeping with its hormonal role providing feedback on bile acid kinetics. A ten-fold difference in serum FGF19 levels in healthy subjects and associations with certain metabolic factors have been demonstrated.^{26, 27} Obese subjects have lower FGF19 fasting values and increases after meals. In our previous work, we demonstrated reduced median FGF19 levels in patients with primary BAD, as defined by SeHCAT or reduced C4 levels,^{10, 13, 14} although others have not found this in overall groups of D-IBS patients defined more broadly.²⁸ A single FGF19 measurement can be influenced by a number of factors, which reduces its diagnostic sensitivity for BAD. We postulated that it is likely some patients with primary BAD have reduced baseline secretion, some could have impairment in the postprandial state, whereas in others, differences in the FGFR4 receptor, the β -Klotho co-receptor or other aspects of the downstream response could also be implicated.^{29–31} In contrast to the variability in individual FGF19 measurements in and between individuals, SeHCAT retention over 7 days is a result of multiple cycles of BA secretion and loss, and so the SeHCAT test integrates an overall loss of bile acids into the colon over time. We therefore used SeHCAT as a more reliable way to define our group of primary BAD patients, and this predicted their response to OCA therapy. Clinical benefits of OCA therapy were not seen in the idiopathic control chronic diarrhoea group with normal SeHCAT retention, although several of these patients had low fasting FGF19 resulting in the median for controls being less than that found in our much larger previous prospective study.¹⁴ In that study, we have discussed other reasons for low fasting FGF19; and presumably other factors such as those related to motility or secretion are predominant causes of diarrhoea in these control patients. Whether SeHCAT is the best predictor for OCA clinical response will need confirmation in larger studies.

Obeticholic acid stimulated increases in blood levels of FGF19, as has been shown before.²⁰ In an ileal explant system with samples from normal subjects, we showed large increases in FGF19 transcripts and protein secretion.¹⁵ We expected to find increased FGF19 levels with OCA in the control patients in this study, but were uncertain whether patients with primary BAD would be able to respond. Their responses, both acutely after the first OCA dose and after 2 weeks treatment, show that in primary BAD, OCA is able to enter the ileal enterocyte and then stimulate FGF19 transcription, leading to median blood level values similar to those found in

healthy controls. FXR agonists may also directly regulate secretion in the colon³², but the present results suggest that induction of ileal FGF19 is the predominant action. Conceivably other downstream effects involving microbial effects on bile acid metabolism, faecal pH or motility could be involved but we have no data to support such actions.

Before this study, we were uncertain whether any clinical response would include changes in stool form, frequency or both. Rescue medication in the form of loperamide was allowed as this increased the ability to recruit patients with unpredictable, socially embarrassing episodes of diarrhoea. To allow for these individual differences, we proposed to use a stool index which combined stool frequency and type but also allowed for loperamide use. The factor chosen (mg loperamide \times 3) equates the use of one standard 2 mg dose of loperamide to a single type 6 stool. The current individual results suggest this is appropriate and further validation is being undertaken. In keeping with the diversity of the various types of disease thought of as IBS, pain was not a common complaint in these primary BAD patients. Their principal problems are frequency, stool form and urgency, which all improved significantly.

In the secondary BAD group, fasting FGF19 levels were significantly lower. We and others have shown that both ileal resection and inflammation are factors underlying this reduction.^{33, 34} The changes in this group were quite variable, but even in this small sample we could demonstrate a significant correlation between the extent of ileal resection and FGF19, C4, BA and a variety of clinical parameters. Therapeutic effects were more significant in the patients with resections shorter than 45 cm. More extensive ileal resections reduce the area available for bile acid (and OCA) uptake, but limitation of the FGF19 response may be the critical factor. Detailed studies involving larger patient cohorts, including measurements of inflammatory responses such as CRP and faecal calprotectin levels, will be of great interest.

Obeticholic acid was well tolerated, consistent with findings in previous clinical trials where the drug has been used in PBC and NAFLD.^{18–21} The increases in total and LDL-cholesterol, along with reduction in triglycerides, found here are very similar to those found before in patients with nonalcoholic fatty liver disease and type 2 diabetes treated with OCA,²⁰ and also in gallstone patients treated with CDCA, the natural FXR agonist.³⁵ This may be an expected consequence of lowering bile acid synthesis. In our overall study population, new bile acid synthesis (as measured by fasting C4 levels) is

reduced by 80% from the pretreatment values and, as bile acid excretion is the main means of cholesterol loss from the body, the 10–20% increase in serum cholesterol levels is not surprising. The time course of these changes and any long-term effects are unknown.

A clear limitation of this study is that recording symptoms in diaries is subjective and could easily have been influenced by placebo-like effects. However, symptomatic responses were considered together with the operator-blinded biochemical measures (FGF19, C4 and BA) and the primary outcome of this proof-of-concept study (fasting FGF19) was a quantitative endpoint. Future trials of the clinical effects of OCA in patients with chronic diarrhoea will utilise a double-blind, randomised, placebo-controlled study design and will include greater numbers of subjects. These studies will give results with a smaller confidence intervals compared to the large confidence intervals found here. The present study also compared symptoms on OCA therapy to symptoms without specific therapy and future trials should include comparisons of the response to OCA with those to bile acid sequestrants.

In conclusion, this proof-of-concept study provides the first evidence that FXR agonists reduce new bile acid synthesis (C4) and postprandial responses, and improve clinical symptom scores in patients with BAD. Targeting FXR to stimulate FGF19 is a novel, rational therapeutic approach for the patients with primary BAD who are currently receiving other treatments for functional diarrhoea or D-IBS, as well as for many Crohn's patients with secondary BAD.

AUTHORSHIP

Guarantor of the article: J. R. F. Walters.

Author contributions: JW, DS, IJ, JN, MP study concept and design; IJ, JN, CV acquisition of data; JW, IJ, JN, CV, MP, DS, analysis and interpretation of data; JW, CV, IJ, JN, statistical analysis; JW, obtained funding and study supervision; JW, IJ, JN, CV, drafting of the manuscript; all authors, critical revision of the manu-

script. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Results.

Data S2. Additional relationships of change in clinical symptoms to FGF19 and bile acids.

Figure S1. FGF19 and bile acid changes during serial sampling over 6 hours.

Table S1. Changes in fasting lipids overall and in each group.

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