Potent stimulation of fibroblast growth factor 19 expression in the human ileum by bile acids

Justine H. Zhang,† Jonathan D. Nolan,† Sarah L. Kennie,† Ian M. Johnston,† Tracy Dew,‡ Peter H. Dixon,§ Catherine Williamson,∥ and Julian R. F. Walters†

†Section of Hepatology and Gastroenterology, Department of Medicine, ‡Obstetric Medicine, Imperial College London and Imperial College Healthcare, London; ∥Clinical Biochemistry, King’s College Hospital, London, United Kingdom

Submitted 18 October 2012; accepted in final form 19 March 2013

Gastroenterology Dept., Imperial College Healthcare, Hammersmith Hospital, Du ileum by bile acids

enterohepatic circulation; bile acid diarrhea

BILE ACIDS (BA) undergo an enterohepatic circulation. They are synthesized in the liver, secreted into the duodenum, reabsorbed in the terminal ileum, and recirculated back to the liver via the portal vein. Synthesis of BA is regulated by a negative feedback mechanism (16), involving in part the enteric hormone fibroblast growth factor 19 (FGF19) in humans (35) and its orthologue FGF15 in mice (13). FGF15/FGF19 are produced in response to the absorption of BA (24) and subsequently act on the liver to inhibit cholesterol 7α-hydroxylase (CYP7A1) (16, 35), the rate-limiting enzyme in the classical pathway of BA synthesis.

In the mouse, FGF15 expression is abundant in ileum (10), and, except for a small amount of FGF15 expression in jejunum, it is undetectable in other tissues. In humans, FGF19 expression was reported in ileum, but was not reliably detected in duodenum (1). Recently, FGF19 expression has also been reported in human gall bladder and biliary tissues (43).

BA have been shown to stimulate the transcription of FGF15/FGF19 through a nuclear receptor, farnesoid X receptor (FXR, NR1H4) in mouse ileum and cultured human cell lines (13–15). Concentrations of the major physiological primary BA, unconjugated chenodeoxycholic acid (CDCA), ranging from 3–100 μmol/l, have been demonstrated to be effective in activating FXR-mediated BA-response elements in reporter genes in human CV-1 cell lines (25, 31, 40). However, FXR activation was only observed with taurine- and glycine-conjugated BA when the cell lines were also transfected with the apical membrane sodium bile acid transporter (ASBT), which would allow uptake of these more polar conjugated BA. FXR is expressed throughout enterohepatic tissues with clearly defined regulatory roles in BA homeostasis in the liver and the ileum (15).

In humans, serum levels of FGF19 are thought to be dependent on the transintestinal flux of BA, based on the serum peaks observed 90–120 min after the postprandial rise in serum BA. In support of this, serum FGF19 was found to decrease after the treatment with a BA-binding resin and to increase upon oral administration of CDCA (24).

Enterocytes in the terminal ileum are specialized to take up conjugated BA such as glyco-chenodeoxycholic acid (GCDCA) and tauro-chenodeoxycholic acid from the intestinal lumen. This function is mediated by the expression of the ileal ASBT. BA then bind to the ileal bile acid-binding protein (IBABP) and are extruded at the basolateral membrane by the organic solute transporter (OST)-αβ heterodimer (8).

Reduced fasting levels of FGF19 have been observed in clinical situations of BA malabsorption (39). This has been shown in patients who have undergone previous ileal resection or ileal Crohn’s disease (20). However, lower FGF19 levels are also observed in patients with the condition known as primary (idiopathic) BA diarrhea (or malabsorption), which, despite its name, is not clearly associated with any malabsorptive defect in most patients (36). We have proposed an alternative mechanism for the diarrhea in this condition. Impaired FGF19 feedback inhibition causes excessive BA synthesis, which exceeds the normal capacity for ileal reabsorption, and so proceeds to induce diarrhea in the colon (39). This is consistent with the observation that the BA pool size is increased in these patients (37).

*J. Zhang and J. Nolan contributed equally to this study.

Address for reprint requests and other correspondence: J. R. F. Walters, Gastroenterology Dept., Imperial College Healthcare, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK (e-mail: julian.walters@imperial.ac.uk).
This study aimed to confirm the expression of FGF19 transcripts in the human ileum and to investigate expression of FGF19 in other parts of the human intestine (10). Using short-term tissue cultures of intestinal biopsies obtained during ileo-colonoscopy, we hypothesized that FGF19 expression would be induced with CDCA and its conjugate GCDCA (31, 32) and proposed to compare this with the other natural primary BA, cholic acid, and the secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA), which differ as FXR agonists. We also included study of the potent synthetic FXR agonist obeticholic acid (OCA) (23, 33). Research into the BA-mediated induction of ileal FGF19 may further elucidate the pathophysiology of gastrointestinal diseases associated with ileal dysfunction, such as Crohn’s disease and primary BA diarrhea, which have previously been associated with reduced circulating levels of serum FGF19 (19, 39).

MATERIALS AND METHODS

Subjects. In total, 42 subjects were recruited from patients undergoing routine ileo-colonoscopy. Subjects gave informed, written consent. All subjects had fasted overnight in preparation for colonoscopy and had received bowel preparation, usually with oral Senna and magnesium citrate. Exclusion criteria were as follows: patients under 18 yr old, patients on anticoagulant or dual-antiplatelet therapy, and patients with microscopic or macroscopic evidence of ileal pathology, as shown on histological examination. Intestinal mucosal biopsies were taken with the same type of disposable biopsy forceps (radial jaw 3; Boston Scientific, Hemel Hempstead, UK), giving standardized mucosal samples. The study was approved by the local Institutional Review Board, the Hammersmith, Queen Charlotte’s, and Chelsea Hospitals’ Research Ethics Committee.

FGF19 baseline expression studies. For FGF19 baseline expression studies, three terminal ileum biopsies were obtained from 11 patients. Additionally, in each patient, 3–6 further biopsies were obtained from up to two of the following colonic sites: cecum, ascending colon, transverse colon, or sigmoid colon. These were stored in RNAlater (Sigma-Aldrich, Gillingham, UK) at −80°C before RNA extraction. Ileal explant studies. We recruited 28 patients for ileal explant studies, one of whom was also a patient in the FGF19 baseline expression studies. Intestinal mucosal biopsies from the terminal ileum for routine histology and research studies, three terminal ileum biopsies were obtained from 11 patients. Additionally, in each patient, 3–6 further biopsies were obtained from up to two of the following colonic sites: cecum, ascending colon, transverse colon, or sigmoid colon. These were stored in RNAlater (Sigma-Aldrich, Gillingham, UK) at −80°C before RNA extraction.

RNA extraction. RNA extraction was performed using a RNeasy Mini kit (Qiagen, Crawley, UK). Reaction volumes of 20 μl were used, containing 1 μg RNA, 1 μl dNTP mix (10 mM), 1 μl random hexamers (50 ng/μl), 2 μl 10× RT buffer, 4 μl MgCl2 (25 mM), 2 μl DTT (0.1 M), 1 μl RNaseOUT (40 U/μl), 1 μl SuperScript II RT (50 U/μl), and diethylpyrocarbonate (DEPC)-treated water. Reverse transcription was performed at 42°C for 50 min. The manufacturer’s guidelines for reverse-transcription controls were included, where SuperScript II RT was replaced with 1 μl DEPC-treated water. Resultant cDNA was treated with 1 μl Escherichia coli RNaseH (2 U/μl) for 20 min at 37°C. Final cDNA was diluted twofold before proceeding with RT-PCR.

Quantitative real-time RT-PCR. Amplification of cDNA was performed using SYBR Green JumpStart Taq Ready Mix (Sigma-Aldrich), working primer stock concentrations of 10 μM, and cDNA volumes of 1 μl per well, in an Applied Biosystems 7900HT Fast cycler (Warrington, UK). Primer sequences and characteristics are shown in Table 1. Singleplex RT-PCR assays were performed in triplicate in 25-μl reaction volumes, using MicroAmp Fast Optical 96-well reaction plates (Applied Biosystems, UK) or in 8 μl reaction volumes using MicroAmp Optical 384-Well reaction plates (Applied Biosystems). Each plate was run with minus reverse-transcriptase controls and PCR controls (1 μl DEPC-treated water added instead of 1 μl cDNA). The thermal cycling program was as follows: 10 min of 95°C (to activate Taq polymerase), then 40 cycles of 15 s at 95°C, 1 min at 60°C, and 1 min at 72°C, ramping at 1.4°C/s between each step of the cycle. Triplicates where differences in cycle time (Ct) values exceed 0.5 Ct were analyzed for outliers, and outliers were removed from calculations. Results were analyzed using the comparative Ct method (also known as the ΔΔCt method) to give fold changes of

Table 1. Primer sequences used in quantitative real-time RT-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human FGF19, forward</td>
<td>CCG TAG CTC TGC CCG ATG GGC</td>
</tr>
<tr>
<td>Human FGF19, reverse</td>
<td>CCA TCT GGG CCG ATC TCC</td>
</tr>
<tr>
<td>Human GAPDH, forward</td>
<td>CCA CCA CTT TGT GAA CTT CAA</td>
</tr>
<tr>
<td>Human GAPDH, reverse</td>
<td>AGG GGA GAT TCA GTC TGG TG</td>
</tr>
<tr>
<td>Human ASBT, forward</td>
<td>CGA CCA AAA AGC AAG GAT CA</td>
</tr>
<tr>
<td>Human ASBT, reverse</td>
<td>CTC AGA CAA ATC AGT AGA</td>
</tr>
<tr>
<td>Human IBABP, forward</td>
<td>TCA GAG ATG GTG GGT GAC AA</td>
</tr>
<tr>
<td>Human IBABP, reverse</td>
<td>TCA CGG GCT CAT AGG TCA</td>
</tr>
<tr>
<td>Human SHP, forward</td>
<td>AGG CAG CAT CCT CTT CAA CAA</td>
</tr>
<tr>
<td>Human SHP, reverse</td>
<td>TTC ACA CAG CAG CAA TGG AG</td>
</tr>
<tr>
<td>Human FXR, forward</td>
<td>AGG ATT TCA GAC TTT GGA CCA TGA</td>
</tr>
<tr>
<td>Human FXR, reverse</td>
<td>TGC CCA AAA AGG AAG CAG CAT</td>
</tr>
<tr>
<td>Human OST-α, forward</td>
<td>AGA TGG CCT GTT CTC CCG CTC</td>
</tr>
<tr>
<td>Human OST-α, reverse</td>
<td>ATT GCT GTT AGC ACA CTT GCT</td>
</tr>
<tr>
<td>Human OST-β, forward</td>
<td>CAG GAG CTT CTT GGA GAG AT</td>
</tr>
<tr>
<td>Human OST-β, reverse</td>
<td>GAC CAT GCT TAT AAT GAC CAG</td>
</tr>
</tbody>
</table>

FGF, fibroblast growth factor; ASBT, apical membrane sodium bile acid transporter; IBABP, ileal bile-acid-binding protein; SHP, short heterodimer partner; FXR, farnesoid X receptor; OST, organic solute transporter.
FGF19 expression relative to the control sample in each patient. GAPDH was used as the endogenous control in each sample to normalize expression.

ELISA. ELISA was used to measure FGF19 protein concentration in supernatant (FGF19 Quantikine ELISA kit; R&D Systems, Minneapolis, MN), according to the manufacturer’s protocols. FGF19 protein values were normalized to the number of biopsies (2–3) incubated in each culture well.

Statistical analyses. Statistical analyses were performed in GraphPad Prism Version 5.04 software (GraphPad Software, San Diego, CA). Nonparametric two-tailed tests (Wilcoxon and Mann-Whitney tests) were used as appropriate. P values <0.05 were considered significant. Correlation of results with clinical variables was tested by calculating Spearman’s rank correlation coefficient.

RESULTS

FGF19 is expressed in human ileum but is undetectable in human colon. We first determined FGF19 expression in the human intestine. Of the 11 ileal samples, all expressed FGF19, with a mean Ct of 29.9, showing lower expression than GAPDH (mean Ct 22.5). Standard error of ΔCt values for FGF19 from ileal samples was 1.141, reflecting that the samples had a low variability of up to twofold difference in FGF19 expression. Inactive Crohn’s disease was present in two patients, but neither of these patients had significantly different ileal FGF19 expression levels from the nine patients without Crohn’s disease. In all the cecal, ascending, transverse, and sigmoid colon samples, FGF19 was either completely undetectable, or expression was at the limits of reliable quantification (Table 2). Expression of FGF19 was less than 0.004 of that seen in the corresponding ileum.

Six-hour incubation does not result in loss of FGF19 mRNA. Groups of ileal samples were incubated for 6 h in the explant system, with either 50 μM CDCA, 50 μM GCDCA, or no BA (unstimulated controls). Unstimulated ileum did not show a difference in FGF19 expression after a 6-h incubation compared with other ileal samples that had not undergone incubation (P = 0.26, Mann-Whitney test) (Fig. 1, left). We confirmed this in four patients with matched samples that were either immediately processed or incubated for 6 h (Fig. 1, right). This shows that incubation of ileum does not result in loss of FGF19 mRNA, providing support for the robustness of this experimental setup.

FGF19 transcript expression in human ileum is strongly induced by CDCA and GCDCA. After 6-h incubation, FGF19 expression increased significantly, with a median induction of 161-fold by 50 μM CDCA and GCDCA at 50 μM CDCA (Fig. 3). A nonlinear, least squares, exponential fit was used to derive the EC50 for studies that included four or five concentrations (5–100 μM). There was no significant difference in induction of FGF19 expression between CDCA and GCDCA-stimulated ileum (P = 0.3, two-tailed Wilcoxon test). Results are expressed graphically in Fig. 2.

The responses of other relevant genes to CDCA and GCDCA at 50 μM (n = 3 - 4) showed median increases for IBABP, OST-α, OST-β and short heterodimer partner (SHP) between 2.4- and 4.0-fold, whereas ASBT and FXR showed little change (Fig. 2). The unstimulated expression of FGF19 was lower (with a high Ct value) than the other genes, but two other FXR-responsive genes differed greatly, with SHP similar to FGF19, but IBABP much higher (with a lower Ct value). Responses to CDCA and GCDCA at 100 μM appeared similar to those at 50 μM (data not shown).

Table 2. Cycle threshold (Ct) values at different biopsy sites

<table>
<thead>
<tr>
<th>Biopsy Site</th>
<th>n</th>
<th>Median Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>8</td>
<td>29.9</td>
</tr>
<tr>
<td>Colon</td>
<td>4</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Ascending</td>
<td>1</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Cecum</td>
<td>1</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Transverse</td>
<td>1</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>6</td>
<td>37.7</td>
</tr>
</tbody>
</table>

Ct values in the gastrointestinal tract show the cycle at which FGF19 amplification occurred (the higher the Ct, the later the amplification, so the lower the gene expression). Ct values >40 cycles signify undetectable gene expression. Ct values >35 cycles are widely considered to signify gene expression at the limits of reliable quantification. n = number of subjects.
incubated with 50 μM CDCA (56 pg/explant, range 8–169) compared with the control explants (7 pg/explant, range 0–46). The median level of FGF19 was also significantly higher (n = 9, P = 0.002, two-tailed Mann-Whitney) in the culture fluid from explants incubated with 50 μM of GCDCA (median 24 pg/explant, range 3–113) compared with the matched control explants (6 pg/explant, range 0–13), (Fig. 5A). There was also a significant correlation between the culture fluid protein and fold changes in mRNA of FGF19 (CDCA: n = 10, r = 0.70, P = 0.0005; GCDCA: n = 9, r = 0.76, P = 0.0003, Spearman’s rank test; Fig. 5B).

The induction of FGF19 mRNA in human ileum by different BA correlates with their relative potencies as FXR agonists. Some ileal explants were incubated with CDCA in parallel with other natural BA (CA, DCA, and LCA) or the synthetic FXR agonist OCA. CDCA and CA were found to have a broadly similar potency at inducing the expression of FGF19 mRNA. The mean induction of FGF19 mRNA expression in explants incubated with 50 μM CA was 81% of that seen with respective paired CDCA incubations, whereas significantly lower induction was found with DCA (40%) and with LCA at 4% (Fig. 6).

After incubations with 1 μM OCA for 6 h, a median fold change of 70 (range 40–258, n = 3) was observed although no induction in FGF19 mRNA expression was observed with 1 μM CDCA (n = 3). In paired incubations involving both 20 μM CDCA and 20 μM OCA, median induction in FGF19 mRNA expression was five times greater with OCA (range 3–45, n = 3). The greater potency of OCA compared with CDCA, apparent at the lower dose of 1 μM, indicates that OCA has a much lower EC50.

Induction of FGF19 expression by BA displays interperson variability. There was variability in different subjects in the magnitude of ileal FGF19 induction after BA stimulation (CDCA-stimulated samples: range 48–939; GCDCA-stimulated samples: range 56–1,065). This may represent differences in equilibrium levels for BA homeostasis between individuals. However, no significant associations between the magnitude of FGF19 induction with age, sex, or stool frequency (≥3/day vs. <3/day) were observed.

**DISCUSSION**

This study has confirmed human ileal expression of FGF19 and has shown for the first time that ileal FGF19 is greatly stimulated by physiological concentrations of BA. Before this study, the effect of BA on FGF19 transcription in human ileum was unknown because no system existed to investigate this. We have now developed an explant system that is functional and does not result in loss of FGF19 mRNA. The potential applications of this system are numerous, as it enables investigation of FGF19 stimulation in human ileum under controlled conditions. This system is more akin to natural human physiological conditions compared with previous systems that used mouse ileum, hepatocytes, or human cell lines. The use of explanted human tissue in this system also allows ileal FGF19 stimulation to be directly linked to subjects, so that ileal FGF19 stimulation may be investigated in different disease states.

We have demonstrated that stimulation of the human ileum with 50 μM CDCA or 50 μM GCDCA induces FGF19 transcript expression in all individuals, with a median induction of ~300-fold. This is greater than the ~80-fold FGF15 induction previously shown in mouse ileum after stimulation with a synthetic FXR agonist GW4064 (15). Species differences and experimental design may have contributed to the magnitude difference.

The large induction in ileal FGF19 expression we have shown is also larger than that previously observed in primary cultures of human hepatocytes (35). In those experiments, control samples had low expression of FGF19, but a dose-dependent increase in...
FGF19 mRNA levels was seen, of about 80–1,200-fold after 6–24-h incubation with CDCA (50 μM) or with GW4064 (1 μM).

We also found that FGF19 is expressed only at low levels in human colon, at the limits of reliable quantification, suggesting that the colon is unlikely to be important in mediating this intestine-liver hormonal signaling for the maintenance of BA homeostasis. FXR, the bile acid-activated nuclear receptor, is expressed in more locations in the gastrointestinal tract than FGF19, including expression in the colon (6). This discrepancy between FGF19 and FXR expression indicates that other factors must also be involved in the transcriptional control of FGF19, to produce high levels in ileum. Other transcription factors that may be candidates for regulating the distribution of FGF19 expression include homeobox transcription factors and GATA4 (3–5, 7, 38).

An FXR-responsive element was first described in the second intron of the FGF19 gene and mediated reporter gene activation in HuH7 cells (13). This is conserved in the mouse Fgf15 gene. More recently, three novel FXR-responsive elements have been described in reporter and binding assays in the S'-flanking region of the FGF19 gene, which are not conserved in the mouse (28). These binding assays used recombinant FXR proteins and specific FXR antibodies, and the FXR response elements activate reporter gene expression when stimulated with the FXR agonists, CDCA or GW4064, to a much greater extent than LCA. LCA alone has a weak effect on a possible pregnane X receptor (PXR) element.

The large 300-fold changes in FGF19 mRNA expression appear to dwarf the 4-fold changes of the other BA-regulated genes investigated in this study. IBABP, OST-α, and OST-β genes have been shown to have FXR-mediated transcriptional responses, which are predicted to lead to an increase BA-ileal fluxes, but their induction in our system was only a fraction of FGF19. SHP, involved in BA-mediated transcription regulation, and previously shown to be highly induced in mouse ileum (15), was also stimulated much less than FGF19. These differences in magnitude suggest that FGF19 may have the dominant role in the negative feedback regulation of the BA enterohepatic circulation, supporting the mouse data on FGF15 (14). ASBT expression has previously been shown to be negatively regulated by BA indirectly through SHP in humans (30), and FGF15/19 in mice and human cell lines can also repress ASBT promoter activation, RNA, and protein expression (34).

One of the main effects of FGF19 is the negative feedback regulation of BA synthesis, and, for this to be achieved, FGF19 transcripts must be translated into protein and released into the portal circulation. We have attempted to look at this by quantifying protein in the culture fluid. Our results reveal significant differences in magnitude between the ~300-fold change in transcript expression and the much lower ~8-fold changes in FGF19 protein. This is could be due to the extra time needed for translation and export of the protein but may reflect some additional regulation of those processes including transcript stabilization, posttranslational modification, secretion, and stability of the protein in the culture fluid.

It is also important to note that the measurements we have made in our explant system are steady-state transcript levels at a particular time, rather than direct measures of FGF19 synthesis or degradation. To get a better idea of the dynamics of FGF19 transcript expression, we conducted limited time course

---

**Fig. 3.** Dose-ranging studies of CDCA induction of FGF19 mRNA. Graph shows pooled data from 10 explant studies that were incubated with 2–5 different concentrations of CDCA in parallel. Concentrations ranged from 1–100 μM. Data points are shown from each individual explant from each individual patient (see data points A–J). Fold changes in mRNA were converted to a percentage of maximum response, which was observed at 50 μM in those studies whose data points are shown in the oval ring and 100 μM for those shown in the rectangular box.

**Fig. 4.** Time courses (n = 4) showing FGF19 expression increased over the course of 6-h incubation with 50 μM CDCA. Incubations with CDCA were for 0.5 h, 1 h, 1.5 h, 2 h, 3 h, or 6 h. Explants were placed into RNA later immediately after incubation. FGF19 expression at 0.5 h and 1 h showed no significant change, at 1.5 h and 2 h was <10-fold, at 3 h was >40-fold, and at 6 h was dramatically higher at several hundred fold. Data are expressed as median fold changes, calibrated to matched unstimulated control samples, relative to GAPDH. Interquartile ranges are shown where appropriate.
experiments, which showed that FGF19 transcript expressions were not increased by 1 h of incubation but, after then, continued to increase up to 6 h. This time course is typical for transcription and shows, in this experimental situation, that, as the length of time of BA exposure increases, more FGF19 transcripts are synthesized than degraded, resulting in a net increase in FGF19 transcript levels.

We studied both unconjugated CDCA and conjugated GCDCA in most experiments because of concerns that GCDCA would only be taken up in cells that expressed ASBT, whereas CDCA could permeate the cell membrane without specific transport systems (25, 31, 40). Expression of ASBT is a key finding in the ileum with its uptake and transcellular flux of BA but is known to be reduced in disease states (17). In our experiments, stimulation was found in ileal explants in all cases with GCDCA, but we have not included any subjects with identified ileal disease.

It is widely acknowledged that the most potent physiological FXR agonist is CDCA, but there has been some ambiguity regarding the relative potency of CA as an FXR agonist (21, 25, 31, 40). Minimal FXR activation was found in nonintestinal CV-1 cells with CA, compared with CDCA and secondary BA (31), and similar findings were reported using human FXR and a reporter gene in HepG2 cells (25). However, other groups have shown that CA is able to stimulate FXR responses (21, 40). Here we have shown that FGF19 is strongly induced with both CDCA and CA and, to a lesser extent, with DCA. Our study used human tissue in a physiological system where BA transport occurs, giving confidence that this system gen-

Fig. 5. Increases in FGF19 protein in culture fluid after bile acid (BA) incubations. A: CDCA ($n = 10$), GCDCA ($n = 9$). FGF19 protein levels were measured by specific ELISA (FGF19 QuantiKine ELISA kit; R&D Systems, Minneapolis, MN) at the end of 6-h incubations. FGF19 protein levels (pg/explant) are higher in the culture fluid from every BA-stimulated explant compared with their respective paired control explant. B: correlation between the culture fluid protein levels and fold changes in mRNA levels of FGF19 CDCA ($n = 10$) and GCDCA ($n = 9$).
cyclic adenosine monophosphate (26). No link has been shown and other tissues, binds certain BA, and increases intracellular receptor, BG37/TGR5, has been found to be expressed in ileum been shown for various BA. The G protein-coupled membrane receptor, BG37/TGR5, has been found to be expressed in ileum and other tissues, binds certain BA, and increases intracellular cyclic adenosine monophosphate (26). No link has been shown between TGR5 activation and downstream FGF15/19 expression or for any other FXR target genes. Interestingly, one study (22) did report a possible "crosstalk" scenario between FGF15 and TGR5 on gallbladder motility. Intestinal TGR5 appears to be linked to glucose and lipid homeostasis (18) rather than BA regulation.

This study successfully confirmed the implications of previous reports (27, 32) showing OCA to be a potent ligand for FXR. OCA strongly induced FGF19 in all subjects at both of the studied concentrations. This supports the potential therapeutically role of OCA as a novel FXR agonist in gastrointestinal disease with defective FXR activation/FGF19 induction patterns. OCA enters the enterohepatic circulation (12) and thus can be used to regulate hepatic BA synthesis. Previous reports have focused on cholestatic diseases (21) and, more recently, metabolic disorders (9, 29), but we would also anticipate a role for OCA in gastrointestinal disorders including primary BA diarrhea.

The individual variability in ileal FGF19 induction by both CDCA and GCDCA indicates different levels of BA homeostatic equilibrium (or disequilibrium). Some people may thus be more susceptible to disorders of BA homeostasis than others. Primary BA diarrhea is likely to be a disease state that is more common in individuals who have disequilibrium of BA homeostasis. Our group has demonstrated that patients with primary BA diarrhea had lower median fasting serum FGF19 levels than controls and proposed that primary BA diarrhea may be due to inadequate FGF19 expression, resulting in impaired negative feedback and consequent overflow of BA into the colon (39). One previous study demonstrated a significant depletion in CA levels in patients with primary BA diarrhea compared with controls (11). This could have been important if our findings had suggested a higher potency for CA in inducing FGF19 compared with CDCA but was not the case, as CDCA and CA were not significantly different. We did not recruit any patients with diagnosed primary BA diarrhea for this study but will be investigating its pathogenesis in further studies, looking at basal and stimulated FGF19 secretion. Studies in patients with ileal inflammatory disease or resection, as in Crohn’s, and perhaps those with other disorders of bile acid regulation, such as gallstone disease, may also be of interest.

In summary, this study has found that FGF19 is highly expressed in human ileum and increases more than 300-fold in 6 h after stimulation with the potent FXR-activating BA, CDCA, and OCA. We have achieved this through an explant system, which in future can be used to investigate the role of FGF19 in disorders of BA homeostasis.

ACKNOWLEDGMENTS

We are grateful to the staff at Hammersmith Hospital Endoscopy Unit who helped in the collection of samples, the patients who volunteered to take part in this study, Sagen Zac-Varghese and Arianna Psichas for assistance in intestinal incubations, and Shadi Abu-Hayyeh, Georgia Papacleovoulou, and Sabina Ravat for assistance in the laboratory. Obeticholic acid was a gift from Intercept Pharmaceuticals, New York, New York.

GRANTS

I. Johnston was funded by the Bardhan Research & Education Trust and J. Nolan by the Broad Medical Research Program. We also acknowledge support from the NIHR Biomedical Research Centre funding scheme and the NIHR/Wellcome Trust Clinical Research Facility at Imperial College Healthcare NHS Trust.
DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


