GASTROINTESTINAL, HEPATOBILIARY, AND PANCREATIC PATHOLOGY

The FXR Agonist Obeticholic Acid Prevents Gut Barrier Dysfunction and Bacterial Translocation in Cholestatic Rats

Len Verbeke,^{*} Ricard Farre,^{†‡} Bert Verbrinnen,^{§¶} Kris Covens,^{||} Tim Vanuytsel,[†] Jan Verhaegen,^{**} Mina Komuta,^{††} Tania Roskams,^{††} Sagnik Chatterjee,^{‡‡} Pieter Annaert,^{‡‡} Ingrid Vander Elst,^{*} Petra Windmolders,^{*} Jonel Trebicka,^{§§} Frederik Nevens,^{*} and Wim Laleman^{*}

From the Division of Liver and Biliopancreatic Disorders,^{*} the Departments of Morphology and Molecular Pathology,^{††} Translational Cell and Tissue Research, and Pharmaceutical and Pharmacological Sciences,^{‡‡} Drug Delivery and Disposition, University Hospitals Leuven, KU Leuven - University of Leuven, Leuven, Belgium; the Translational Research Center for Gastrointestinal Disorders,[†] the Experimental Laboratory Immunology,[§] the Department of Molecular and Vascular Biology,^{||} and the Laboratory of Clinical Bacteriology and Mycology,^{**} KU Leuven - University of Leuven, Leuven, Belgium; the Center for Biomedical Research, Network for Liver and Digestive Diseases (CIBERehd),[‡] Instituto de Salud Carlos III, Barcelona, Spain; the Department of Life Sciences,[¶] Thomas More Kempen, Geel, Belgium; and the Department of Internal Medicine I,^{§§} University of Bonn, Bonn, Germany

Accepted for publication
October 2, 2014.

Address correspondence to Len Verbeke, M.D., Ph.D., Division of Liver and Biliopancreatic Disorders, University Hospitals Leuven, KU Leuven—University of Leuven, B-3000 Leuven, Belgium. E-mail: len.verbeke@med.kuleuven.be.

Bacterial translocation (BTL) drives pathogenesis and complications of cirrhosis. Farnesoid X-activated receptor (FXR) is a key transcription regulator in hepatic and intestinal bile metabolism. We studied potential intestinal FXR dysfunction in a rat model of cholestatic liver injury and evaluated effects of obeticholic acid (INT-747), an FXR agonist, on gut permeability, inflammation, and BTL. Rats were gavaged with INT-747 or vehicle during 10 days after bile-duct ligation and then were assessed for changes in gut permeability, BTL, and tight-junction protein expression, immune cell recruitment, and cytokine expression in ileum, mesenteric lymph nodes, and spleen. Auxiliary *in vitro* BTL-mimicking experiments were performed with Transwell supports. Vehicle-treated bile duct–ligated rats exhibited decreased FXR pathway expression in both jejunum and ileum, in association with increased gut permeability through increased claudin-2 expression and related to local and systemic recruitment of natural killer cells resulting in increased interferon- γ expression and BTL. After INT-747 treatment, natural killer cells and interferon- γ expression markedly decreased, in association with normalized permeability selectively in ileum (up-regulated claudin-1 and occludin) and a significant reduction in BTL. *In vitro*, interferon- γ induced increased *Escherichia coli* translocation, which remained unaffected by INT-747. In experimental cholestasis, FXR agonism improved ileal barrier function by attenuating intestinal inflammation, leading to reduced BTL and thus demonstrating a crucial protective role for FXR in the gut–liver axis. (*Am J Pathol* 2015, 185: 409–419; <http://dx.doi.org/10.1016/j.ajpath.2014.10.009>)

Intestinal bacterial translocation (BTL) is defined as the migration of viable micro-organisms from the gut lumen toward the mesenteric lymph nodes (MLNs) and extra-intestinal sites such as the peritoneal cavity.¹ BTL has been documented in both animal and human cirrhosis, and its occurrence is considered detrimental in the natural history of a patient with chronic liver disease, because it either preludes hepatic decompensation or propagates further liver impairment, often culminating in multiorgan failure and death.^{2–9} Also, translocation of pathogen-associated molecular patterns such as endotoxins has been shown to drive progression of and

complications in liver disease in cirrhotic patients.⁶ Ample evidence from observational studies in cirrhotic patients has shown that BTL increases with increasing degree of

Supported by Ph.D. fellowships (L.V. and T.V.) and a postdoctoral fellowship (R.F.) from the Fund for Scientific Research—Flanders (FWO Vlaanderen) and German Research Foundation (Deutsche Forschungsgemeinschaft) grant SFB TRR57 project 18 (J.T.). W.L. and F.N. are senior clinical investigators for the Fund for Scientific Research—Flanders (FWO Vlaanderen).

Disclosures: Intercept Pharmaceuticals provided the obeticholic acid compound (INT-747).

hepatocellular failure, rather than the degree of portal hypertension, although animal studies have shown BTL to occur also in conditions of acute and chronic portal hypertension.^{3,5} Especially rapidly progressive jaundice appears to coincide with infection, because histological features of intrahepatic cholestasis such as ductular bilirubinostasis predict the development of septicemia and worsen prognosis in patients with acute decompensating cirrhosis.^{10,11} Furthermore, obstructive jaundice has been found to promote BTL in humans, irrespective of the presence of advanced liver disease.¹² Taken together, these findings suggest a strong association between BTL and cholestasis in liver disease.^{10–12}

Another pivotal element in BTL is increased intestinal permeability, which facilitates migration of bacteria and bacterial products such as endotoxins across the gut wall.^{13–16} *In vivo*, several studies have confirmed increased permeability in cirrhotic patients by means of functional assays such as [⁵¹Cr]-EDTA or lactulose–mannitol tests.^{17,18} Recently, knowledge has been accumulating on the complexity, selectivity, and dynamic character of intestinal epithelial barrier. A distinction has been made between at least two key functional pathways: a high-capacity pore pathway allowing the passage of small ions and a low-capacity leak pathway determining large-molecule permeability, the first being regulated mainly by claudins and the latter by occludin and tight-junction proteins of the zonula occludens family.¹⁹ Furthermore, this tight junction–mediated permeability appears to be regulated by the local expression of pro- and anti-inflammatory interleukins, among which interferon gamma (IFN- γ) and tumor necrosis factor- α (TNF- α) have received considerable interest.^{20–24}

The farnesoid X-activated receptor (FXR) has emerged as a promising target for numerous hepatobiliary and gastrointestinal disorders. In addition to its main function as a bile acid–responsive transcription regulator of hepatic bile metabolism, its expression and functionality has recently been documented in intestine (especially ileum), immune cells, and endothelial tissue.^{25–28} FXR knockout mice not only exhibit pronounced hepatic inflammation and fibrosis, but they also develop an inflammatory bowel disease–like phenotype, with increased intestinal inflammation and permeability and eventually BTL.²⁵

We hypothesized that, in a rat model of cholestatic liver injury, dysfunctional intestinal FXR signaling serves as the central molecular switch driving BTL, facilitated by increased

intestinal permeability and orchestrated by intestinal inflammation, which can be restored by means of gavage of the highly selective and potent FXR agonist obeticholic acid [INT-747; provided by David Shapiro and Luciano Adorini (Intercept Pharmaceuticals, San Diego, CA)].

Materials and Methods

Animal Models

All animal experiments were performed according to guidelines of the local ethics committee. Male Wistar rats (Janvier Labs, Le Genest St Isle, France) weighing 200 to 250 g were divided into five experimental groups. The first group served as untreated healthy controls ($n = 28$). In the second group ($n = 6$), toxic compensated cirrhosis was induced through administration of thioacetamide in their drinking water for 18 weeks, as described previously.²⁹ The other three groups underwent ligation of the common bile duct (BDL, $n = 51$) and were treated with vehicle, 5 mg/kg UDCA, or 5 mg/kg INT-747 (kindly provided by Intercept Pharmaceuticals) every 2 days during the period of BDL, based on dose-finding studies in which increasing mortality was observed with increasing doses of INT-747: 3/7 animals at 5 mg/kg per day and 7/7 animals at 30 mg/kg per day. Mortality was related to hepatic INT-747 accumulation after blockage of enterohepatic circulation. Animals were sacrificed at 10 days after BDL.

RT-PCR of SHP, Tight-Junction Proteins, and Proinflammatory Cytokines in Ileum

The relative expression of the FXR downstream signaling molecule small heterodimer partner (SHP), tight-junction proteins (claudin-1, claudin-2, occludin, and ZO-1), and the proinflammatory cytokines TNF- α and IFN- γ was determined by RT-PCR. For this purpose, total RNA was extracted from jejunum and ileum samples ($n = 5$ to 8 per group) in TRIzol reagent (Invitrogen; Life Technologies, Carlsbad, CA; Merelbeke, Belgium) and reverse transcribed. cDNA was amplified by PCR by use of rat-specific primers (Table 1). Primers were designed using sequence data and nucleotide BLAST software from the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov/nucleotide>) and were manufactured by TIB

Table 1 Primers Used for Analysis of mRNA Expression Levels in Rat Ileum

Protein (Gene)	Forward	Reverse
SHP (<i>Nrob2</i>)	5'-CTTGAGCTGGGTCCCAAGGA-3'	5'-CTAGCTGGGTACCAGGGCTC-3'
Claudin-1 (<i>Cldn1</i>)	5'-ATTGGCATGAAGTGCATGAG-3'	5'-CCACTAATGTCGCCAGACCT-3'
Claudin-2 (<i>Cldn2</i>)	5'-GGCTATTAGGCACATCGAT-3'	5'-TGGCACCAACATAAGAAGCTT-3'
ZO-1 (<i>Tjp1</i>)	5'-CCCTCTGATCATTTCCACACA-3'	5'-TACATGTCATTGCTTGGTGC-3'
Occludin (<i>Ocln</i>)	5'-ATGTCTGTGAGGCCCTTTTGA-3'	5'-TTTAGACATGCGCTCTTCCT-3'
TNF- α (<i>Tnf</i>)	5'-GATCGGTCCCAACAAGGAGG-3'	5'-GCTTGGTGGTTTGCTACGAC-3'
IFN- γ (<i>Ifng</i>)	5'-TATCTGGAGGAAGTGGCAAAAG-3'	5'-TGCGATTTCGATGACACTTATGT-3'

MolBiol (Berlin, Germany). All samples were run in duplicate with a LightCycler 480 systems (Roche Applied Science, Mannheim, Germany; Indianapolis, IN). Samples were normalized to the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (*Hprt*), and fold-change expression was compared with the healthy control group using the $2^{-\Delta\Delta CT}$ method, as described previously.³⁰

Detection of BTL in Ascites Fluid and MLNs

To evaluate the presence of viable translocated bacteria, 1 mL of ascites fluid (peritoneal lavage in healthy controls) was aspirated from the abdomen, and MLNs were dissected under fully sterile conditions. The MLNs were then homogenized and cultured on multiple different growth plates: Columbia Agar-based blood agar with 5% horse blood (Thermo Fisher Scientific; Oxoid, Basingstoke, UK; Waltham, MA), Wilkens–Chalgren anaerobe broth, MacConkey agar, mannitol salt agar, Sabouraud agar with chloramphenicol, thioglycollate medium, and tryptone soy broth. MLNs lining the large bowel were divided into four anatomical regions, proximal to distal, and were scored according to the number of bacterial strains detected in culture.

Assessment of Intestinal Permeability in Ussing Chambers

Full-thickness intestinal tissue taken from mid-jejunum and terminal ileum ($n = 5$ rats per group) was mounted in triplicate in 3-mL modified Ussing chambers as described previously, with a 0.096-cm² area of tissue exposed.¹⁵ The mucosal side was exposed to 10 mmol/L mannitol and the serosal side to 10 mmol/L glucose in Krebs–Ringer bicarbonate buffer, and the samples were maintained at 37°C and carbogenated with 95%/5% O₂/CO₂ at all times, to preserve tissue viability throughout the experiment.³¹ Gut permeability was assessed by trans-epithelial electrical resistance (TEER) and dextran permeability. TEER was measured after induction of bipolar constant-current pulses of 50 μ A every 1 minute, and average values were acquired from the first 60 minutes of measurement after a 30-minute stabilization period and expressed as $\Omega \cdot \text{cm}^2$. Macromolecular permeability was determined by the trans-epithelial paracellular passage of fluorescein isothiocyanate (FITC)-labeled 20-kDa dextran (Sigma-Aldrich, Diegem, Belgium; St. Louis, MO) from the mucosal to the serosal side. For this experiment, the average fluorescence levels in the serosal buffer samples, taken every 30 minutes after addition of 3 mg/mL labeled dextran to the mucosal buffer, were analyzed with a Fluoroskan Ascent microplate fluorometer (Thermo Fisher Scientific).

Analysis of Immune Cells and Activation Markers in Spleen and MLNs by Flow Cytometry

Single-cell suspensions of white blood cells from spleen and MLNs ($n = 4$ rats per group) were obtained by use of 100 μ mol/L cell strainers (BD Biosciences, Erembodegem,

Belgium; San Jose, CA). Total cell count was performed using an ABX Micros 60 automated cell counter (Horiba Instruments, Irvine, CA). Per sample, 2.5×10^6 cells were stained in phosphate-buffered saline containing 0.5% bovine serum albumin for 30 minutes at 4°C in the dark and were fixed in 1% formaldehyde solution. The following rat-specific antibodies were used for flow cytometry: CD45–FITC and CD45–phycoerythrin-Cy7 for white blood cells and CD45RA–allophycocyanin-Cy7 for B-cells (BD Biosciences); CD103–FITC for dendritic cells (BioLegend, Dedham, MA); NKR-PIA–phycoerythrin for natural killer cells (Invitrogen; Life Technologies); and CD11b/c2–peridinin chlorophyll protein complex (PerCP)–eFluor 710 for macrophages, CD4–allophycocyanin for helper T cells, and CD8a–phycoerythrin-Cy7 for cytotoxic T cells (eBioscience, Vienna, Austria; San Diego, CA). The following activation markers were assessed on CD11b/c2⁺ cells: CD80–allophycocyanin (Invitrogen; Life Technologies) and CD86–FITC (eBioscience). Flow cytometry was performed on a FACSCanto II cytometer (BD Biosciences).

Assessment of Direct Antibacterial Potential of INT-747 and UDCA

The bacteriostatic or bactericidal effects of both INT-747 and UDCA were assessed *in vitro* by Mueller–Hinton broth detecting culture growth inhibition at concentrations ranging from 1 to 256 μ g/mL in cultured strains of *Klebsiella oxytoca*, *K. pneumoniae*, *Staphylococcus aureus*, *S. warneri*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Enterococcus faecalis*, *Proteus mirabilis*, and *Citrobacter freundii*.

Detection and Quantification of Bile Acids in Blood and Urine

Samples of heparinized blood plasma and urine were collected from BDL rats and healthy controls at sacrifice ($n = 5$ per group). In each sample, deoxycholic acid (DCA), taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDCA), and glycochenodeoxycholic acid (GCDCA) were detected and quantified by use of a liquid chromatography–mass spectrometry system (Thermo Fisher Scientific, Breda, The Netherlands) as described previously.³²

In Vitro Assessment of Relationships between FXR, Intestinal Permeability, and BTL Under Inflammatory Conditions

Confluent monolayers of the human intestinal epithelial cell line Caco-2 were grown on Snapwell permeable support membranes (Corning Life Sciences, Tewksbury, MA) until steady-state TEER was achieved, as described previously.³³ Cells were grown in modified Eagle's medium (Life Technologies, Bleiswijk, The Netherlands) enriched with 10%

bovine serum albumin, 1 mmol/L sodium pyruvate, 2 mmol/L glutamine and 1% antibiotic solution (Invitrogen; Life Technologies). Epithelial permeability was evaluated by TEER measurement with an EndOhm resistance meter (World Precision Instruments, Sarasota, FL); the trans-epithelial flux of 4-kDa FITC-labeled dextran (Sigma-Aldrich, Belgium) from the apical to the basolateral compartment was analyzed with a Fluoroskan Ascent microplate fluorometer (Thermo Fisher Scientific). Translocation of Texas Red–labeled *E. coli* bacteria (K-12 strain; Life Technologies) was assessed with a Fluoroskan Ascent microplate fluorometer (Thermo

Fisher Scientific). At the start of experiments, the apical compartment was replaced with medium containing 2×10^5 *E. coli* particles per milliliter and 100 $\mu\text{mol/L}$ dextran replenished with either vehicle (control conditions), 10 ng/mL IFN- γ (eBioscience), and/or 10 $\mu\text{mol/L}$ of the FXR agonist INT-747. TEER and basolateral samples were analyzed every 24 hours during a 96-hour period. Average ΔTEER from the start to 48 hours and 72 hours, compared with control conditions, and average passage of dextran and bacteria between 48 and 96 hours, was compared between groups, based on experiments by Madara and Stafford.³³ The experiments were repeated at least three times under identical conditions.

Statistical Analysis

Statistical data were tested for equal variance and normal distribution. Data were all normally distributed and therefore were expressed as means $\pm$ SEM and were subjected to parametrical statistics. When comparing two unpaired groups, the Student's *t*-test was applied; for comparison of multiple unpaired groups, a one-way analysis of variance was applied. If positive, a subsequent pairwise comparison was performed by means of Bonferroni *t*-testing. *P* values below 0.05 were considered statistically significant. Statistical analysis was performed with GraphPad Prism software version 6.02 (GraphPad Software, La Jolla, CA).

Results

FXR Deactivation in Intestine of Cholestatic Rats Is Associated with Increased Gut Permeability and Intestinal BTL but Can Be Prevented by Selective FXR Reactivation in the Ileum

The median number of translocated bacterial strains dropped from 4 to 2 in MLNs of BDL rats after treatment with

Figure 1 Effects of FXR agonism on BTL, gut permeability, and FXR pathway in the several experimental groups. **A:** The median number of bacterial strains isolated from MLN was reduced from 4 to 2 in INT-747–treated, but not in UDCA-treated, BDL rats. No BTL was observed in healthy control or TAA toxic cirrhotic rats. **B:** TEER decreased in both jejunum and ileum of vehicle-treated BDL rats, compared with healthy controls, reflective of increased intestinal permeability. After treatment with INT-747, a selective restoration of TEER was observed in the ileum, but not in the jejunum, of BDL rats. **C:** INT-747 induced a selective reduction of dextran passage in the ileum of BDL rats, compared with both vehicle-treated BDL and healthy control rats, consistent with decreased intestinal permeability. **D:** Expression of the FXR downstream receptor SHP was reduced in the jejunum and ileum of vehicle-treated BDL rats, compared with healthy controls, and was significantly increased in the ileum, but not the jejunum, of INT-747–treated BDL rats. Data are expressed as means $\pm$ SEM. Rats per group: *n* = 5 [**A** (BDL+INT-747, BDL+UDCA), **B**, and **C**]; *n* = 6 [**A** (Control, TAA, BDL)]; *n* = 8 (**D**). **P* $\leq$ 0.05, ***P* $\leq$ 0.01, and ****P* $\leq$ 0.001. BDL, bile-duct ligated; BTL, bacterial translocation; FXR, farnesoid X-activated receptor; INT-747, obeticholic acid; MLN, mesenteric lymph nodes; SHP, small heterodimer partner; TAA, thioacetamide; TEER, transepithelial electrical resistance; UDCA, ursodeoxycholic acid.

INT-747 ($P \leq 0.01$) (Figure 1A). This was associated with sterile ascites in 5/6 INT-747-treated BDL rats, compared with only 1/6 vehicle-treated BDL rats. INT-747 significantly increased survival (19/19), compared with vehicle treatment (11/16) in BDL rats ($P = 0.01$). UDCA treatment did not affect either BTL or survival. No BTL was observed in thioacetamide-treated rats (an anicteric model of toxic cirrhosis and portal hypertension) (Figure 1A). TEER decreased in the jejunum of vehicle-treated BDL rats, compared with healthy controls (49 ± 2 versus $58 \pm 2 \Omega \cdot \text{cm}^2$; $P \leq 0.01$), and in the ileum (48 ± 1 versus $60 \pm 4 \Omega \cdot \text{cm}^2$; $P < 0.05$), consistent with increased intestinal permeability (Figure 1B). With FXR agonist treatment, selective restoration of TEER (back to healthy control values) was observed in the ileum of BDL rats, but not in the jejunum (Figure 1B). The transepithelial passage of dextran remained unaffected in the jejunum of INT-747-treated BDL rats; in the ileum, however, macromolecular passage was clearly decreased ($14 \pm 2 \text{ pmol} \cdot \text{cm}^2$), compared with both vehicle-treated BDL rats ($33 \pm 8 \text{ pmol} \cdot \text{cm}^2$) and healthy control rats ($27 \pm 5 \text{ pmol} \cdot \text{cm}^2$; $P \leq 0.05$) (Figure 1C). These findings corroborate the selective reduction of intestinal permeability in the ileum by FXR stimulation (Figure 1, B and C). In the gut of vehicle-treated BDL rats, the expression of the FXR downstream target receptor small heterodimer partner (SHP) was markedly reduced, compared with healthy controls, reflective of a deactivated FXR pathway, which appeared to be reactivated in the ileum by administration of INT-747, but not in the jejunum ($P \leq 0.05$) (Figure 1D), which parallels the apparent selective functional restoration of the ileum mentioned earlier.

Restoration of Intestinal Permeability in the Ileum of BDL Rats by INT-747 Relates to Altered Expression of the Tight-Junction Proteins Claudin-1 and Occludin

In both the jejunum and ileum of vehicle-treated BDL rats, expression of the pore-forming tight-junction protein claudin-2 increased significantly, compared with healthy controls ($P \leq 0.01$) (Figure 2B), whereas the expression of the counterbalancing pore-closing claudin-1 remained unaffected (Figure 2A). By contrast, INT-747-treated BDL rats exhibited a significant selective ileal increase in expression of pore-closing claudin-1, compared with both vehicle-treated BDL and healthy control rats ($P \leq 0.02$) (Figure 2A). Furthermore, in the ileum of INT-747-treated BDL rats the expression of occludin, one of the chief inhibitory regulators of the leak pathway, was significantly up-regulated compared with vehicle-treated BDL rats ($P = 0.02$) (Figure 2C). Ileal expression of ZO-1 was significantly up-regulated in INT-747-treated BDL rats, compared with healthy control rats (fold change 2.19 ± 0.34 versus 1.01 ± 0.05 ; $P \leq 0.01$); however, the difference failed to reach statistical significance in comparison with vehicle-treated counterparts ($P = 0.12$).

Figure 2 Effects of FXR agonism on tight-junction protein expression in jejunum and ileum of BDL rats. **A** and **B**: In the jejunum and ileum of vehicle-treated BDL rats, a significant increase in expression of pore-forming tight-junction protein claudin-2 (**B**) was observed, compared with healthy controls consistent with increased permeability. In INT-747-treated BDL rats, this was counterbalanced by an increased expression of pore-closing claudin-1 (**A**), which was absent in vehicle-treated BDL rats and the jejunum of INT-747-treated BDL rats. **C**: INT-747-treated BDL rats exhibited an increased expression of occludin-1 in the ileum, compared with their vehicle-treated counterparts. Data are expressed as means $\pm$ SEM. $n = 8$ rats per group. $*P \leq 0.05$, $**P \leq 0.01$.

The FXR Agonist INT-747 Induces a Strong Intestinal and Systemic Anti-Inflammatory Response in BDL Rats, Associated with Decreased Local Expression of IFN- γ in the Ileum

The average spleen/body weight ratio was significantly reduced in BDL rats treated with INT-747, compared with vehicle-treated BDL rats and even with healthy control rats (0.24 ± 0.04 versus 0.39 ± 0.03 and 0.36 ± 0.04 , respectively; $n = 4$ rats per group; $P \leq 0.05$). This was associated with an overall decrease in total white blood cell count in spleen of BDL rats receiving INT-747, compared with vehicle ($1.24 \pm 0.54 \times 10^7$ versus $2.93 \pm 0.45 \times 10^7$; $P \leq 0.05$) (Figure 3A), and similarly in MLNs ($0.38 \pm 0.08 \times 10^7$

Figure 3 Evaluation of local and systemic inflammation in BDL rats treated with the FXR agonist INT-747. **A** and **B**: Flow cytometry assessment of white blood cells in spleen (**A**) and MLN (**B**) homogenates. A significant increase in NKCs and macrophages was observed in BDL rats, compared with healthy controls. In BDL rats treated with INT-747, recruitment of NKCs was again reduced to healthy control values. **C**: Flow cytometry illustration of spleen homogenate from representative control, vehicle-treated, and INT-747-treated BDL rats. The NKC fraction increased after BDL, and then decreased after INT-747 treatment. **D**: Ileal expression of IFN- γ as assessed by RT-PCR increased after BDL, and then decreased to below healthy control values after INT-747 treatment. **E**: After BDL, expression of the activation marker CD80 increased on macrophages in both spleen and MLNs, as assessed by flow cytometry, and was unaffected by FXR agonist treatment. Data are expressed as means $\pm$ SEM. Rats per group: $n = 4$ (**A–C**); $n = 8$ (**D** and **E**). * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. APC, allophycocyanin; IFN- γ , interferon- γ ; MHCII, major histocompatibility complex II; CD161, natural killer cell surface marker; PE, phycoerythrin.

versus $1.04 \pm 0.15 \times 10^7$; $P \leq 0.01$) (Figure 3B). In terms of white blood cell subtypes in spleen, vehicle-treated BDL rats exhibited significantly increased numbers of both natural killer cells (NKCs) [$2.3 \pm 0.4 \times 10^6$ (8.2% of viable cells)] and macrophages [$8.6 \pm 1.6 \times 10^6$ (27.3%)], compared with

healthy controls [$1.4 \pm 0.2 \times 10^6$ NKCs (7.4%) and $2.2 \pm 0.9 \times 10^6$ macrophages (11%); $P \leq 0.05$] (Figure 3A). Similarly, in MLNs, vehicle-treated BDL rats exhibited significantly increased numbers of both NKCs [$6.3 \pm 0.1 \times 10^4$ (0.7%) of viable cells] and macrophages

[$12.8 \pm 2.0 \times 10^4$ (1.2%)], compared with healthy controls [$2.6 \pm 0.1 \times 10^4$ NKC (0.3%) and $4.2 \pm 0.8 \times 10^4$ macrophages (0.6%); $P \leq 0.05$] (Figure 3B). Treatment with INT-747 induced a reduction in dendritic cells, cytotoxic and helper T cells, and B cells in both spleen and MLN of BDL rats (data not shown), but the decreased number of NKCs was particularly notable, in both spleen [$0.8 \pm 0.4 \times 10^6$ (6.1% of viable cells)] and MLNs [$2.0 \pm 0.7 \times 10^4$ (0.7%)], compared with vehicle-treated BDL rats ($P \leq 0.05$) (Figure 3, A–C).

In the ileum of vehicle-treated BDL rats, expression of IFN- γ , a cytokine produced mainly by NKCs, increased significantly; with INT-747 treatment, however, IFN- γ expression decreased to below healthy control values ($P \leq 0.01$) (Figure 3D). The functionality of macrophages in the ileum, as reflected by expression of activation markers such as CD80 (Figure 3E), was unaffected by INT-747 treatment; CD86 expression and local production of TNF- α in the ileum were also unaffected (data not shown).

The FXR Agonist INT-747 Does Not Significantly Alter Composition of the Bile Acid Pool during 10 Days of BDL

In BDL rats, we observed an important increase in circulating bile acids in blood, especially GDCA and CDCA, along with increased urinary excretion of DCA, TDCA, CDCA, TCDCA, and GCDCA, all consistent with cholestasis. After FXR administration, however, neither blood and urinary bile acid concentrations nor conjugation of bile acids was significantly affected (Supplemental Table S1).

IFN- γ Induces *E. coli* BTL *in Vitro*, Which Remains Unaffected by Local FXR Stimulation

The functional relevance of IFN- γ was substantiated *in vitro* by incubating a human intestinal epithelial monolayer with 10 ng/mL IFN- γ . This induced increased *E. coli* BTL, compared with healthy control (26 ± 8 versus 8 ± 4 colony-forming units (CFU)/mL) ($P \leq 0.03$) (Figure 4). This translocation remained unaffected by simultaneous incubation with 10 μ mol/L INT-747 (26 ± 8 versus 41 ± 20 CFU/mL; $P = 0.43$) (Figure 4). Intestinal permeability, as assessed by TEER and dextran passage, also remained unaffected after INT-747-addition to an IFN- γ -exposed intestinal epithelium of Caco-2 cells (data not shown).

UDCA and INT-747 Have No Clear Direct Bacteriostatic Potential *in Vitro*

In Mueller–Hinton broth, no inhibition of *in vitro* growth of the bacterial strains was obtained for concentrations of ≤ 128 μ g/mL of either UDCA or INT-747. At 256 μ g/mL, both agents showed similar complete inhibition of bacterial growth in all strains tested; however, these concentrations

are not obtained *in vivo* and classically are considered to reflect dose-related toxicity.

Discussion

BTL is considered a harmful event in the natural history of chronic liver disease, because it either preludes hepatic decompensation or acts as a pacemaker to further liver insufficiency and multiorgan failure. Both intestinal bacterial overgrowth and a leaky gut are considered to be key events, but to date the available clinical approaches toward prevention and treatment of BTL have been limited to intestinal decontamination by means of antibiotics.³⁴ The increasing rate of treatment failures, due to numerous infections with Gram-positive and multiresistant microorganisms, points to the need for novel therapies in treatment and prevention of BTL and of spontaneous bacterial peritonitis, the most common infection in patients with cirrhosis.^{35–37}

From this perspective, we focused on FXR, a bile acid-responsive nuclear transcription factor that is crucial as a chief regulator of hepatic bile acid, lipid, and carbohydrate metabolism.^{25,28,38–41} Interestingly, FXR is also highly expressed in the intestine, so the effects extend beyond the liver. More specifically, the pivotal role of FXR in the preservation of intestinal homeostasis is increasingly recognized, along with its involvement in the gut–liver axis. An example is the recent finding that FXR-dependent signaling from the gut through fibroblast growth factor-15 (FGF-15) protects mice against cholestasis.⁴² FXR deficiency has therefore been linked to increased intestinal permeability and BTL in non–liver-related gastrointestinal disorders, such as inflammatory bowel disease.^{25,26} However, whether FXR dysfunction also relates to BTL in chronic liver disease (and also what are the underlying pathophysiological mechanisms) remains unclear.

Figure 4 *In vitro* assessment of BTL. Incubation of a human intestinal epithelial Caco-2 monolayer with 10 ng/mL IFN- γ induced increased BTL of *Escherichia coli*, which was not affected by simultaneous treatment with the FXR agonist INT-747. * $P \leq 0.05$.

Figure 5 Schematic representation of the proposed pathophysiology. In BDL rats, after treatment with the FXR agonist INT-747, recruitment of both NKC and the NKC-derived proinflammatory cytokine IFN- γ was markedly reduced, leading to restoration of intestinal epithelial integrity through up-regulation of the tight-junction proteins claudin-1, occludin, and ZO-1 and thus preventing translocation of bacteria across the gut wall. MLCK, myosin light-chain kinase; NKC, natural killer cells.

In the present study, we confirmed intestinal FXR pathway deficiency in a rat model of chronic cholestatic liver injury. This deficiency was associated with high rates of BTL and increased intestinal permeability, which in turn was related to a disproportional up-regulation of the pore-forming tight-junction protein claudin-2 throughout the small bowel.

Based on these findings, we then aimed to reactivate the FXR pathway by oral administration of the first-in-class highly potent and selective FXR agonist 6- α -ethyl-chenodeoxycholic acid (obeticholic acid; INT-747), a semisynthetic bile acid derivative whose value has recently been authenticated in phase II and III clinical trials for nonalcoholic fatty liver disease and primary biliary cirrhosis.^{28,41,43–45} To discriminate between FXR-specific and general bile acid-related effects, INT-747-gavaged rats were compared with rats gavaged with equal amounts of ursodeoxycholic acid (UDCA), a bile acid similar in molecular structure to INT-747 but without FXR agonist properties.⁴³ Interestingly, after treatment with INT-747, a reactivation of the FXR pathway was observed only in the ileum and not the jejunum of BDL rats, which paralleled selective ileal restoration of intestinal integrity and coincided with a marked reduction in BTL, thus suggesting the ileum as a primary source for BTL in liver disease. The finding that FXR is expressed mainly in the ileum is in accord with previous reports by others and is not surprising, given the pivotal role of FXR in regulation of enterohepatic reabsorption of bile acids and ileal clustering of gut immunity.^{46,47} Although increased gut permeability in the jejunum was equally associated with reduced SHP expression, it appeared unresponsive to FXR agonists, which thus failed to restore jejunal permeability. The reason remains unclear, but it might relate to the effects of FXR agonists on the

immune system, which is more abundant in the ileum than in the jejunum; alternatively, it might relate to a non-FXR-dependent mechanism.

Functionally, restored ileal integrity was related to a marked increase in ileal expression of pore-closing claudin-1, but the leak pathway also was targeted by INT-747, via up-regulation of occludin and ZO-1. Thus, it seems that the relative overexpression of the culprit pore-forming tight-junction protein claudin-2 is overcome by these compensatory protective mechanisms. Given that the tight junction-mediated regulation of paracellular intestinal permeability is highly regulated by local expression of pro- and anti-inflammatory cytokines, we investigated both the recruitment and functionality of all relevant components of the innate and adaptive immunity in this context.^{15,19,48,49} Vehicle-treated BDL rats exhibited a significant increase in NKC and macrophages in both spleen and MLNs. Of additional interest in this context is the observation that the increase in NKC in vehicle-treated BDL rats was concurrent with a marked and specific increase of ileal expression of IFN- γ . IFN- γ is a pro-inflammatory cytokine that is predominantly expressed by NKC as a part of the innate immune response and is considered particularly important in intestinal permeability, for several reasons. First, IFN- γ induces increased permeability in cultured intestinal epithelial monolayers, allowing the passage of *E. coli*-derived lipopolysaccharide *in vitro*.^{20,23} Second, recent findings suggest that IFN- γ also enhances the increased permeability induced by other interleukins such as TNF- α .²¹ Third, IFN- γ promotes the internalization of epithelial tight-junction proteins and, fourth, IFN- γ also appears to promote passage of bacteria such as *Escherichia coli* via a transcellular route.^{22,24} After treatment with INT-747 restored FXR activity, we observed a general

anti-inflammatory response with, in particular, a remarkable decrease in the number of NKCs, both locally in the MLNs and systemically in the spleen, consistent with findings by others on the hepatic level.⁵⁰ As a logical consequence, IFN- γ levels (in parallel with the decreased number of NKCs in the INT-747-treated BDL group) were equally suppressed in the gain-of-function group, strengthening the arguments in favor of a fundamental role for IFN- γ in intestinal permeability.

Given that we were observed absent BTL in an anicteric model of toxic compensated cirrhosis with portal hypertension, but clearly present BTL in a model of short-term cholestatic injury, the next question is whether the observed effects are mediated either directly by obeticholic acid on the intestine or indirectly through an altered bile acid metabolism.⁴³ Interestingly, FXR stimulation did not significantly change the increased bile acid pool or its composition, did not increase the conjugation of primary or secondary bile acids, and did not affect urinary excretion. Thus, taken together with the fact that the observed effects on BT and permeability were obtained in the absence of bile acids and that INT-747 and UDCA lack inhibition on bacterial growth *in vitro* on Mueller–Hinton broth, the findings support a primary and bile acid-independent effect of INT-747 on the intestinal barrier.

This then leads to the question of whether the observed restoration of ileal epithelial integrity is a direct protective effect on the enterocyte with secondary decrease in inflammation or an indirect effect primarily through its NF- κ B-mediated anti-inflammatory potential in intestinal immune cells, similar to effects observed by others in animal models of inflammatory bowel disease.^{26,51,52} For this purpose, we performed auxiliary *in vitro* experiments on a human monolayer of intestinal epithelial Caco-2 cells endowed with enterocyte-like properties, including the formation of tight junction-mediated cell-to-cell adhesions, and we assessed changes in permeability and the translocation of *E. coli*, a species frequently translocated in BDL rats in the present study. We confirmed the IFN- γ -mediated increase in BTL *in vitro*, but BTL and permeability remained unaffected by costimulation with INT-747. This finding favors indirect anti-inflammatory properties of INT-747, rather than a primary direct effect on the intestinal barrier (Figure 5). In addition to decreased NKCs and IFN- γ levels, the observed anti-inflammatory effect was further substantiated by the fact that in INT-747-treated BDL rats, the average spleen weight (as well as the total number of white blood cells in spleen and MLNs) decreased even to below the level of healthy controls.

Although we cannot exclude an additional direct effect of INT-747 on gut microbiota, we do not expect this to alter the observed beneficial effects of treatment with the FXR agonist INT-747 in BDL rats, because we observed absence of direct inhibition by INT-747 of *in vitro* bacterial growth of strains frequently translocated in the present study. This argues against a functionally relevant effect of FXR agonists on intestinal bacterial overgrowth and gut microbiota in general.

A final question might be whether the observed effects on BTL in this model of cholestatic injury relate to the broader context of human cirrhosis, which is primarily toxic or viral in origin. First, here we have demonstrated absent BTL in a model of compensated toxic cirrhosis, comparable to the human condition.⁵ Second, others have recently shown that INT-747 reduces BTL in carbon tetrachloride-intoxicated rats, a representative model of decompensated toxic cirrhosis.⁵³ Interestingly, a similar reactivation of FXR in the ileum of INT-747-treated cirrhotic rats was found to be associated with marked reduction of ileal inflammatory mediators such as IL-17 and IFN- γ ,⁵³ similar to what was found in the present study. This suggests not only that the positive effects of FXR agonism on BTL extend beyond cholestatic liver injury, but also that they can be extrapolated to the broader context of decompensated cirrhosis, with high comparability for the mechanism of action.

In conclusion, we have demonstrated that the FXR agonist INT-747 exerts a protective effect on gut and liver in a rat model of cholestasis. This relates to an anti-inflammatory effect, mainly through decreased NKC-mediated IFN- γ expression in the ileum, resulting in restored epithelial integrity and a subsequent decrease in intestinal BTL. This approach should be further explored in patients with chronic liver disease.

Acknowledgments

We thank David Shapiro and Luciano Adorini (Intercept Pharmaceuticals) for kindly providing the obeticholic acid compound.

L.V. was responsible for the study concept and design, acquisition of data analysis and interpretation of data, obtaining funding, drafting of the manuscript, and statistical analysis; R.F., B.V., K.C., T.V., J.V., M.K., T.R., J.T., S.C., and P.A. provided technical support, acquisition of data, and critical revision of the manuscript; I.V.E. and P.W. provided administrative and technical support and acquisition of data; F.N. performed analysis and interpretation of data, critical revision of the manuscript for important intellectual content, and obtaining funding; and W.L. provided study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, statistical analysis, obtaining funding, and study supervision.

Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2014.10.009>.

References

1. Berg RD, Garlington AW: Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. *Infect Immun* 1979, 23:403–411

2. Garcia-Tsao G, Lee FY, Barden GE, Cartun R, West AB: Bacterial translocation to mesenteric lymph nodes is increased in cirrhotic rats with ascites. *Gastroenterology* 1995, 108:1835–1841
3. Garcia-Tsao G, Albillos A, Barden GE, West AB: Bacterial translocation in acute and chronic portal hypertension. *Hepatology* 1993, 17:1081–1085
4. Llovet JM, Bartolí R, Planas R, Cabré E, Jimenez M, Urban A, Ojanguren I, Arnal J, Gassull MA: Bacterial translocation in cirrhotic rats. Its role in the development of spontaneous bacterial peritonitis. *Gut* 1994, 35:1648–1652
5. Cirera I, Bauer TM, Navasa M, Vila J, Grande L, Taurá P, Fuster J, García-Valdecasas JC, Lacy A, Suárez MJ, Rimola A, Rodés J: Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001, 34:32–37
6. Verbeke L, Nevens F, Laleman W: Bench-to-beside review: acute-on-chronic liver failure - linking the gut, liver and systemic circulation. *Crit Care* 2011, 15:233
7. Laleman W, Verbeke L, Meersseman P, Wauters J, van Pelt J, Cassiman D, Wilmer A, Verslype C, Nevens F: Acute-on-chronic liver failure: current concepts on definition, pathogenesis, clinical manifestations and potential therapeutic interventions. *Expert Rev Gastroenterol Hepatol* 2011, 5:523–537. quiz 537
8. Bellot P, García-Pagán JC, Francés R, Abalde JG, Navasa M, Pérez-Mateo M, Such J, Bosch J: Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* 2010, 52:2044–2052
9. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V; CANONIC Study Investigators of the EASL–CLIF Consortium: Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013, 144:1426–1437. 1437.e1–e9
10. Katoonizadeh A, Laleman W, Verslype C, Wilmer A, Maleux G, Roskams T, Nevens F: Early features of acute-on-chronic alcoholic liver failure: a prospective cohort study. *Gut* 2010, 59:1561–1569
11. Altamirano J, Miquel R, Katoonizadeh A, Abalde JG, Duarte-Rojo A, Louvet A, Augustin S, Mookerjee RP, Michelena J, Smyrk TC, Buob D, Leteurtre E, Rincón D, Ruiz P, García-Pagán JC, Guerrero-Marquez C, Jones PD, Barritt AS, Arroyo V, Bruguera M, Bañares R, Ginès P, Caballería J, Roskams T, Nevens F, Jalan R, Mathurin P, Shah VH, Bataller R: A histologic scoring system for prognosis of patients with alcoholic hepatitis. *Gastroenterology* 2014, 146:1231–1239. e1–e6
12. Kuzu MA, Kale IT, Cöl C, Tekeli A, Köksoy C: Obstructive jaundice promotes bacterial translocation in humans. *Hepatogastroenterology* 1999, 46:2159–2164
13. Pascual S, Such J, Esteban A, Zapater P, Casellas JA, Aparicio JR, Girona E, Gutiérrez A, Carnices F, Palazón JM, Sola-Vera J, Pérez-Mateo M: Intestinal permeability is increased in patients with advanced cirrhosis. *Hepatogastroenterology* 2003, 50:1482–1486
14. Zuckerman MJ, Menzies IS, Ho H, Gregory GG, Casner NA, Crane RS, Hernandez JA: Assessment of intestinal permeability and absorption in cirrhotic patients with ascites using combined sugar probes. *Dig Dis Sci* 2004, 49:621–626
15. Du Plessis J, Vanheel H, Janssen CE, Roos L, Slavik T, Stivaktas PI, Nieuwoudt M, van Wyk SG, Vieira W, Pretorius E, Beukes M, Farré R, Tack J, Laleman W, Fevery J, Nevens F, Roskams T, Van der Merwe SW: Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* 2013, 58:1125–1132
16. Reiberger T, Ferlitsch A, Payer BA, Mandorfer M, Heinisch BB, Hayden H, Lammert F, Trauner M, Peck-Radosavljevic M, Vogelsang H; Vienna Hepatic Hemodynamic Lab: Non-selective beta-blocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. *J Hepatol* 2013, 58:911–921
17. Thalheimer U, De Iorio F, Capra F, del Mar Lleo M, Zuliani V, Ghidini V, Tafi MC, Caburlotto G, Gennari M, Burroughs AK, Vantini I: Altered intestinal function precedes the appearance of bacterial DNA in serum and ascites in patients with cirrhosis: a pilot study. *Eur J Gastroenterol Hepatol* 2010, 22:1228–1234
18. Ramos AR, Matte U, Goldani HA, Oliveira OL, Vieira SM, Silveira TR: Intestinal permeability assessed by 51Cr-EDTA in rats with CCl4-induced cirrhosis. *Arq Gastroenterol* 2010, 47:188–192
19. Shen L, Weber CR, Raleigh DR, Yu D, Turner JR: Tight junction pore and leak pathways: a dynamic duo. *Annu Rev Physiol* 2011, 73:283–309
20. Watson CJ, Hoare CJ, Garrod DR, Carlson GL, Warhurst G: Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. *J Cell Sci* 2005, 118:5221–5230
21. Wang F, Graham WV, Wang Y, Witkowski ED, Schwarz BT, Turner JR: Interferon-gamma and tumor necrosis factor-alpha synergize to induce intestinal epithelial barrier dysfunction by up-regulating myosin light chain kinase expression. *Am J Pathol* 2005, 166:409–419
22. Bruewer M, Utech M, Ivanov AI, Hopkins AM, Parkos CA, Nusrat A: Interferon-gamma induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *FASEB J* 2005, 19:923–933
23. Clark E, Hoare C, Tanianis-Hughes J, Carlson GL, Warhurst G: Interferon gamma induces translocation of commensal *Escherichia coli* across gut epithelial cells via a lipid raft-mediated process. *Gastroenterology* 2005, 128:1258–1267
24. Smyth D, McKay CM, Gulbransen BD, Phan VC, Wang A, McKay DM: Interferon-gamma signals via an ERK1/2-ARF6 pathway to promote bacterial internalization by gut epithelia. *Cell Microbiol* 2012, 14:1257–1270
25. Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W: Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology* 2008, 48:1632–1643
26. Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, Klomp LW, Siersema PD, Schipper ME, Danese S, Penna G, Laverny G, Adorini L, Moschetta A, van Mil SW: Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* 2011, 60:463–472
27. Li J, Wilson A, Kuruba R, Zhang Q, Gao X, He F, Zhang LM, Pitt BR, Xie W, Li S: FXR-mediated regulation of eNOS expression in vascular endothelial cells. *Cardiovasc Res* 2008, 77:169–177
28. Verbeke L, Farre R, Trebicka J, Komuta M, Roskams T, Klein S, Vander Elst I, Windmolders P, Vanuytsel T, Nevens F, Laleman W: Obeticholic acid, a farnesoid-X receptor agonist, improves portal hypertension by two distinct pathways in cirrhotic rats. *Hepatology* 2014, 59:2286–2298
29. Laleman W, Vander Elst I, Zeegers M, Servaes R, Libbrecht L, Roskams T, Fevery J, Nevens F: A stable model of cirrhotic portal hypertension in the rat: thioacetamide revisited. *Eur J Clin Invest* 2006, 36:242–249
30. Schmittgen TD, Livak KJ: Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008, 3:1101–1108
31. Wallon C, Braaf Y, Wolving M, Olaison G, Söderholm JD: Endoscopic biopsies in Ussing chambers evaluated for studies of macromolecular permeability in the human colon. *Scand J Gastroenterol* 2005, 40:586–595
32. Chatterjee S, Bijsmans IT, van Mil SW, Augustijns P, Annaert P: Toxicity and intracellular accumulation of bile acids in sandwich-cultured rat hepatocytes: role of glycine conjugates. *Toxicol In Vitro* 2014, 28:218–230
33. Madara JL, Stafford J: Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. *J Clin Invest* 1989, 83:724–727

34. Wiest R, Krag A, Gerbes A: Spontaneous bacterial peritonitis: recent guidelines and beyond [Erratum appeared in Gut 2012, 61:636]. Gut 2012, 61:297–310
35. Ariza X, Castellote J, Lora-Tamayo J, Girbau A, Salord S, Rota R, Ariza J, Xiol X: Risk factors for resistance to ceftriaxone and its impact on mortality in community, healthcare and nosocomial spontaneous bacterial peritonitis. J Hepatol 2012, 56:825–832
36. Navasa M, Follo A, Llovet JM, Clemente G, Vargas V, Rimola A, Marco F, Guarner C, Forné M, Planas R, Bañares R, Castells L, Jimenez De Anta MT, Arroyo V, Rodés J: Randomized, comparative study of oral ofloxacin versus intravenous cefotaxime in spontaneous bacterial peritonitis. Gastroenterology 1996, 111:1011–1017
37. Umgelter A, Reindl W, Miedaner M, Schmid RM, Huber W: Failure of current antibiotic first-line regimens and mortality in hospitalized patients with spontaneous bacterial peritonitis. Infection 2009, 37: 2–8
38. Vanwijnngaerden YM, Wauters J, Langouche L, Vander Perre S, Liddle C, Coulter S, Vanderborgh S, Roskams T, Wilmer A, Van den Berghe G, Mesotten D: Critical illness evokes elevated circulating bile acids related to altered hepatic transporter and nuclear receptor expression. Hepatology 2011, 54:1741–1752
39. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B: Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 2009, 89: 147–191
40. Fickert P, Fuchsbichler A, Moustafa T, Wagner M, Zollner G, Halilbasic E, Stöger U, Arrese M, Pizarro M, Solís N, Carrasco G, Caligiuri A, Sombetzki M, Reisinger E, Tsybrovskyy O, Zatloukal K, Denk H, Jaeschke H, Pinzani M, Trauner M: Farnesoid X receptor critically determines the fibrotic response in mice but is expressed to a low extent in human hepatic stellate cells and periductal myofibroblasts [Erratum appeared in Am J Pathol 2010, 176:2581]. Am J Pathol 2009, 175:2392–2405
41. Mudaliar S, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, Adorini L, Sciacca CI, Clopton P, Castellote E, Dillon P, Pruzanski M, Shapiro D: Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. Gastroenterology 2013, 145: 574–582.e1
42. Modica S, Petruzzelli M, Bellafante E, Murzilli S, Salvatore L, Celli N, Di Tullio G, Palasciano G, Moustafa T, Halilbasic E, Trauner M, Moschetta A: Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. Gastroenterology 2012, 142:355–365. e1–4
43. Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, Parks DJ, Willson TM: 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. J Med Chem 2002, 45: 3569–3572
44. Vanwijnngaerden YM, Langouche L, Derde S, Liddle C, Coulter S, van den Berghe G, Mesotten D: Impact of parenteral nutrition versus fasting on hepatic bile Acid production and transport in a rabbit model of prolonged critical illness. Shock 2014, 41:48–54
45. Poupon R: Ursodeoxycholic acid and bile-acid mimetics as therapeutic agents for cholestatic liver diseases: an overview of their mechanisms of action. Clin Res Hepatol Gastroenterol 2012, 36(Suppl 1):S3–S12
46. Neimark E, Chen F, Li X, Shneider BL: Bile acid-induced negative feedback regulation of the human ileal bile acid transporter. Hepatology 2004, 40:149–156
47. Thomas AM, Hart SN, Kong B, Fang J, Zhong XB, Guo GL: Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine [Erratum appeared in Hepatology 2010, 52:402]. Hepatology 2010, 51:1410–1419
48. Vanheel H, Vicario M, Vanuytsel T, Van Oudenhove L, Martinez C, Keita AV, Pardon N, Santos J, Söderholm JD, Tack J, Farré R: Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. Gut 2014, 63:262–271
49. Vanuytsel T, van Wanrooy S, Vanheel H, Vanormelingen C, Verschueren S, Houben E, Salim Rasoel S, Tóth J, Holvoet L, Farré R, Van Oudenhove L, Boeckxstaens G, Verbeke K, Tack J: Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. Gut 2014, 63:1293–1299
50. Mencarelli A, Renga B, Migliorati M, Cipriani S, Distrutti E, Santucci L, Fiorucci S: The bile acid sensor farnesoid X receptor is a modulator of liver immunity in a rodent model of acute hepatitis. J Immunol 2009, 183:6657–6666
51. Adorini L, Pruzanski M, Shapiro D: Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. Drug Discov Today 2012, 17:988–997
52. McMahan RH, Wang XX, Cheng LL, Krisko T, Smith M, El Kasmí K, Pruzanski M, Adorini L, Golden-Mason L, Levi M, Rosen HR: Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. J Biol Chem 2013, 288:11761–11770
53. Ubeda M, Borrero MJ, Lario M, Muñoz L, Conde E, Rodríguez M, Lledó L, García-Bermejo L, Álvarez-Mon M, Albillos A: The farnesoid X receptor agonist, obeticholic acid, improves intestinal antibacterial defense and reduces gut bacterial translocation and hepatic fibrogenesis in CCl4-cirrhotic rats with ascites. Abstracts of the International Liver Congress 2014; 49th Annual meeting of the European Association for the Study of the Liver. April 9–13, 2014, London, UK. J Hepatol 2014;61(1 Suppl):S63 (abstract O153)