Enhancement of liver regeneration and liver surgery

Olthof, P.B.

Link to publication

Creative Commons License (see https://creativecommons.org/use-remix/cc-licenses):
Other

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 12

Obeticholic acid accelerates liver regeneration following portal vein embolization in a rabbit model

PB Olthof*, F Huisman*, FG Schaap, KP van Lienden, RJ Bennink, RF van Golen, M Heger, J Verheij, PL Jansen, SW Olde Damink, TM van Gulik
*equal author contribution

British Journal of Surgery 2016
ABSTRACT

Introduction: The bile salt-activated transcription factor farnesoid X-receptor (FXR) is a key mediator of proliferative bile salt signaling, an event implicated in the early phase of compensatory liver growth. The aim of this study was to evaluate the effect of a potent FXR agonist (obeticholic acid, OCA) on liver growth following portal vein embolization (PVE).

Methods: Thirty-six rabbits were randomized between daily oral gavage with OCA (10mg/kg) or vehicle starting 7 days before PVE, and continued until 7 days post-PVE. PVE of the cranial liver lobes was performed using polyvinyl alcohol particles and coils at day 0. Caudal liver volume (CLV) was analyzed by CT volumetry at days -7, -1, +3 and +7. Liver function (mebrofenin uptake) was determined using hepatobiliary scintigraphy. Additional parameters analyzed were plasma transaminase levels, histological scoring of H&E- and Ki67-stained liver sections.

Results: Three days after PVE, the increase of CLV in the OCA group was 2.2-fold greater than in controls (56.1±20.3% vs. 26.1±15.4, \( P<0.001 \)), and this increase remained significantly higher 7 days after PVE (+1.5 fold, \( P=0.02 \)). The increase in caudal liver function at day +3 was greater in OCA treated animals (+1.2 fold, \( P=0.02 \)). The number of Ki67-positive hepatocytes was 1.6-fold higher in OCA-treated animals 3 days after PVE (\( P<0.05 \)). Plasma transaminase levels and histology were similar in both groups.

Discussion: OCA accelerated liver regeneration after PVE in a rabbit model. OCA treatment could therefore increase the efficacy of PVE and, thereby, prevent liver failure after major liver resection and increase resectability.
INTRODUCTION
The liver is the only human organ that is able to regenerate following injury or loss of tissue mass. This regenerative capacity is essential in liver surgery, as it allows liver resection of up to 70% of liver mass with acceptable morbidity and mortality. However, clinical outcomes correlate to amount and function of remnant liver and its ability to regenerate. Enhancing liver regeneration could be instrumental to overcome these surgical limitations. The search for pharmacological interventions to stimulate liver regeneration is still ongoing and has not yet resulted in a clinical application.

Recently, bile acids (BAs) have been identified as early mediators of liver regeneration through activation of the nuclear BA receptor FXR (farnesoid X receptor). Bile flow is essential for liver regeneration after partial hepatectomy, and disruption of the enterohepatic circulation causes delayed liver regeneration. This is attributed to loss of signaling via FXR, as Fxr deficient mice display delayed regeneration following partial hepatectomy. Although both hepatic and intestinal Fxr are involved in liver regeneration after partial hepatectomy, intestinal FXR activation might be the primary mediator via the production and portal release of mitogenic and bile salt homeostatic fibroblast growth factor 19 (FGF19, mouse orthologue: Fgf15). Absence of Fgf15 impaired regeneration and increased mortality after partial hepatectomy in mice. Fgf15 acts through the hepatocyte Fgfr4 receptor to regulate BA synthesis and stimulate regenerative signaling.

Recently, potent FXR agonists have been developed which might stimulate liver regeneration by way of the central action of FXR in bile salt homeostasis, as well as direct induction of proliferative genes. Obeticholic acid (OCA) is a semi-synthetic BA analogue with around 100-fold increased potency for human FXR compared to the most potent endogenous bile salt agonist (i.e. chenodeoxycholic acid). OCA represses hepatic BA synthesis by downregulating the BA synthetic enzyme CYP7A1, limits hepatocyte BA uptake by downregulation of BA importer NTCP, and stimulates basolateral and canalicular BA export by upregulating OSTα/β and BSEP, respectively. These actions all contribute to maintain low intrahepatic BA levels, thereby preventing BA hepatotoxicity and promoting normal progression of liver regeneration after partial hepatectomy. Furthermore, OCA might directly stimulate hepatocellular proliferation through expression of cell cycle regulatory transcription factor Foxm1b. Alternatively, OCA-induced expression of FGF19/Fgf15 in the ileum might exert similar BA lowering in hepatocytes through FGFR4 and promotion of several regeneration inducing pathways.

It was hypothesized that OCA stimulates liver regeneration when applied during portal vein embolization (PVE), a preoperative procedure to increase future liver remnant (FLR)
volume.\textsuperscript{12} PVE induces a 37-62\% FLR volume increase during 29-34 days,\textsuperscript{12,13} The relatively long interval between PVE and liver resection in patients might necessitate chemotherapy to limit tumor progression in the embolized segments.\textsuperscript{14} On the other hand, the regenerative response following PVE is not always sufficient to proceed to safe liver resection.\textsuperscript{12} Enhancing PVE-induced liver regeneration has a clear clinical benefit as it renders more patients eligible for surgery.

The aim of the study was to examine the effect of OCA on liver regeneration in a standardized rabbit model of PVE.\textsuperscript{15} Rabbits were randomized between OCA or vehicle treatment, starting 7 days before PVE and lasting for 7 days after PVE. Liver growth was assessed by CT scanning, hepatobiliary scintigraphy and image analysis, and by immunohistochemistry for the proliferative marker Ki67. Liver injury was examined by measurement of plasma transaminases and assessment of liver histology.

This study shows that OCA accelerates the volume gain of the non-embolized segment over the first 7 days after PVE by increasing liver volume and function. OCA has thus potential as pharmacologic intervention to enhance liver regeneration.

**MATERIALS AND METHODS**

**Animals**
The animal ethics and welfare committee of the Academic Medical Center approved all experimental protocols (BEX35AC and BEX35AD). Thirty-six New Zealand White rabbits (Charles River, Gennat, France) with a mean weight of 2941(±267) gram were allowed to acclimatize for 1 week before inclusion in the experiments. Rabbits were housed in groups in a temperature-controlled room with a 12 h light/dark cycle and ad libitum access to water and standard chow. Animal experiments were reported according to the ARRIVE guidelines.

**Experimental design**
Six groups of six rabbits were planned for PVE. Animals were randomly allocated to either daily obeticholic acid (a generous gift from Intercept Pharmaceuticals, New York, NY) treatment (10 mg/kg in 1\% methyl cellulose) or vehicle (1\% methyl cellulose, Sigma Aldrich, Zwijndrecht, the Netherlands) via oral gavage (1.5 mL for a 3 kg animal). Treatment was started 7 days before PVE, and continued until sacrifice at 3 or 7 days after PVE.
Portal vein embolization
Animals were anesthetized by subcutaneous injection of ketamine (25 mg/kg, Nimatek, Eurovet, Bladel, The Netherlands) and medetomidine (0.2 mg/kg, Dexdomitor, Orion, Espoo, Finland). Isoflurane 2% (Forene; Abbott Laboratories, Kent, United Kingdom) mixed with O₂/air (1:1, 3 L/min) was used to maintain anesthesia. Preoperative analgesia consisted of buprenorphine (0.03 mg/kg, Temgesic, Reckitt Benckiser Healthcare, Hull, United Kingdom). Antibiotic prophylaxis consisted of subcutaneous injection of enrofloxacin (0.2 mg/kg body weight, Baytril, Bayer Healthcare, Berlin, Germany).

PVE was performed as described previously. Following a midline laparotomy, a branch of the inferior mesenteric vein was cannulated using an 18G catheter (Hospira Venisystems, Lake Forest, IL, US). Under digital subtraction portography a Renegade 3F microcatheter (Boston Scientific, Natick, MA) with a Transend-ex 0.36 mm × 182-cm guide wire (Boston Scientific) was positioned in the main portal branch to the cranial liver lobes. Polyvinyl alcohol particles (90–180 µm and 300–500 µm in diameter, Cook, Bloomington, IN) and 2 fibered platinum coils (4.0 and 6.0 mm; Boston Scientific) were infused through the catheter to occlude the portal branches to the cranial lobes. PVE was confirmed by portography, and the mesenteric vein was closed using a ligature. The abdomen was closed in two layers. Baytril was administered daily for 3 days following PVE.

CT volumetry
Multiphase CT scans (Brilliance 64, Philips, Eindhoven, the Netherlands) were performed at day -7, -1, +3, and +7. Animals (n=18 per treatment group) were anesthetized and a 22G catheter was placed in the lateral ear vein. A baseline scan was made and 3 mL of contrast solution (Visipaque, GE Healthcare, Waukesha, WI) was injected. Arterial, portal and venous phase scans were made after 15, 30 and 45 seconds, respectively. Volumetric analysis was performed on 3D reconstruction of 5-mm axial slices using manual delineation. Caudal liver volume (CLV) and total liver volume (TLV) were determined, and increase in CLV was calculated using the following formula:

\[
\% \text{ increase CLV} = \frac{(CLV_{day \times} - CLV_{baseline})}{CLV_{baseline}} \times 100\%
\]

The same formula was used to calculate the decrease in cranial liver volume (CrLV; CrLV = TLV-CLV). Increase in CLV and decrease in CrLV was calculated using day -1 values as baseline. Note that for graphical purposes, changes in CLV and CrLV are displayed starting at day 0 (time point of PVE). Regeneration rate was calculated using the formula:

\[
\text{Regeneration rate (\%/day)} = \frac{\text{increase CLV}_{day \times}}{\text{day x}}
\]
In order to validate CT volumetric data, the volumetric measurements at sacrifice were correlated to the actual liver weight (precision scale, Sartorius, Göttingen, Germany) at sacrifice (figure S1).

**Hepatobiliary scintigraphy**
Liver function was assessed using hepatobiliary scintigraphy (HBS) with $^{99m}$Tc-labeled (2,4,6 trimethyl-3-bromo) iminodiacetic acid ($^{99m}$Tc-mebrofenin, Bridatec, GE Healthcare, Eindhoven, the Netherlands) at days -7, -1, +3 and +7. Rabbits (n=6 per treatment group) were anesthetized and positioned on an imaging table with the liver and heart positioned under a large field-of-view single photon emission computed tomography (SPECT/CT) camera (Siemens Symbia T16). Regions of interest were drawn around the left ventricle for blood pool readings, around the entire liver for total liver uptake, and around the caudal liver lobe (Figure S2). Per rabbit, a dose of 50 MBq $^{99m}$Tc-mebrofenin was administered via a lateral ear vein directly before the start of acquisition.

The geometric mean of datasets of the anterior and posterior cameras was used for analysis. Hepatic $^{99m}$Tc-mebrofenin uptake rate was calculated as an increase of $^{99m}$Tc-mebrofenin uptake over two minutes, corrected for perfusion. Total liver uptake was represented by the total hepatic $^{99m}$Tc-mebrofenin uptake rate, and calculated as a percentage of the injected dose per minute. The fractional $^{99m}$Tc-mebrofenin uptake rate was calculated for the caudal liver lobe, based of distribution of segmental activity and was corrected for baseline measurements at $t = -7$ days. Correction for day -7 was chosen to exclude effects of OCA treatment on mebrofenin uptake before PVE. HBS measurements were optimized in earlier pilot experiments (data not shown).

**Histology**
Liver tissue (left lateral and caudal lobes) was fixed in buffered formalin for 48 h, and subsequently dehydrated and embedded in paraffin. Four micron sections of liver tissue were cut and stained with standard hematoxylin and eosin stain. Sections were scored for lobular and portal inflammation according to table S1. Additionally, liver sections were stained with Ki67 antibodies to quantify hepatocyte proliferation, and hematoxylin counterstain as described previously.$^{16, 17}$ Ki67-positive hepatocytes were counted in a total of five high power fields per animal, by a hepatopathologist (JV) blinded to the group allocation. Liver histology was assessed at day 3 and 7 for n=6 per treatment group.

**Clinical Chemistry**
Serum alanine transaminase (ALT), amino aspartate aminotransferase (AST), gamma-glutamyl transferase (γGT), and alkaline phosphatase (ALP) were determined by the Department of
Clinical Chemistry (Academic Medical Center, Amsterdam, The Netherlands) using a Cobas 8000 modular analyzer (Roche, Basel, Switzerland) (n=12 per treatment group).

**PCR**

Total RNA was isolated from terminal ileum and (non)embolized liver lobes using Tri Reagent (Ambion). Following treatment with DNase I (Promega, Leiden, the Netherlands), 750 ng total RNA was converted to cDNA using the iSCRIPT cDNA synthesis kit (BioRad, Veenendaal, the Netherlands). Quantitative RT-PCR was performed on an IQ5 Cycler using SYBR Green chemistry (SYBR Green MasterMix, BioRad) and cDNA equivalent to 7.5 ng total RNA as template. Expression levels were calculated using LinReg software, and normalized to the geometric mean of *Rplp0*, *Hprt* and *Gapdh*. Primer sequences are provided in Supplemental Table S2. Predicted amplicon size was checked by agarose gel electrophoresis. Transcript analysis was performed using tissue obtained at day +3 and +7 (n=6 per treatment group).

**Statistical analysis**

Differences in non-parametric data between groups were tested using Mann-Whitney U-tests or Kruskal-Wallis tests. Effects of OCA on CLV increase or CrLV decrease were analyzed using Mann-Whitney U-tests on values obtained by area under the curve analysis. Differences between plasma laboratory values were analyzed using two-way ANOVA. Correlations were tested using Spearman’s rank correlation coefficient. All statistical analysis was performed using Graphpad Prism 6.0 (Graphpad Inc, La Jolla, CA).

**RESULTS**

**Obeticholic acid accelerates liver regeneration following portal vein embolization**

Seven days of OCA pretreatment was chosen to ensure adequate tissue levels of OCA at the time of PVE. In mice, diet enriched with cholic acid induced spontaneous liver growth in an FXR dependent manner. Therefore it was first assessed whether liver growth was induced by OCA by analyzing TLV in the pre-treatment period (day -7 until day -1). At both time points, TLV corrected for body weight was similar between groups, and similar between the time points in both groups (Figure 1A). Thus, OCA did not induce spontaneous liver growth. Next, the effect of OCA on PVE-induced liver growth was examined. The PVE procedure was tolerated well, although 2 animals died before the end of the experiments due to a technical complication during induction of anesthesia and mesenteric vein cannulation. Hence, liver volumetry data at day 3 after PVE was available for 34 animals. At this time point, 12 animals were sacrificed, leaving 22 animals for volumetric assessment at day 7 after PVE.
Liver hypertrophy of the caudal lobe was assessed 3 and 7 days after PVE and expressed as percent increase from day -1 values. At 3 days after PVE, the volume of the caudal non-embolized liver had increased 2.2-fold in the OCA group compared to the vehicle controls (Figure 1B, 56.1 ± 20.3% vs. 26.1 ± 15.4%, P < 0.001). At day 7 after PVE, the increase in caudal liver volume remained 1.5-fold higher in OCA-treated animals (102.0 ± 38.2 vs. 67.6 ± 17.7 %, P = 0.02), indicating that OCA accelerates liver regeneration in the first seven days. The decrease in liver volume of the embolized segments was similar between the two groups (Figure 1C). This suggests that OCA has direct effects on regeneration and does not affect atrophy of the embolized cranial lobes.

The volume gain induced by OCA is only relevant when actual liver function is also increased. This was assessed by quantifying mebrofenin uptake, a marker for liver function that is increasingly being used in clinico-surgical practice. Total liver uptake of mebrofenin was similar at days –1, and remained stable after PVE in both groups (Figure 1D). The contribution of the non-embolized caudal liver lobe (CLF share) to total liver mebrofenin uptake increased in both groups 3 and 7 days after PVE. However, the increase was greater in OCA-treated animals 3 days after PVE (44.5 ± 5.4% vs. 36.0 ± 3.7%, P = 0.02), indicating that OCA promotes increase of functional capacity of the non-embolized liver lobe (Figure 1E). To examine whether increased volume gain in OCA treated animals reflect hypertrophy or hyperplasia, liver sections were stained for the proliferation marker Ki67, which is not expressed in quiescent hepatocytes. Increased numbers of Ki67-positive hepatocytes were apparent in the OCA group at day +3, with similar numbers in the groups at day +7. (Figure 1E). Increased hepatocellular proliferation thus underlies augmented liver growth in OCA treated animals at day 3 after PVE.

To exclude changes in body weight as a confounding factor in the liver volume calculations, body weight of the animals was measured daily. Body weight decreased after PVE in both groups to a similar extent (Figure 1G). Body weight gain prior to PVE was similar in both groups, indicating that OCA was well tolerated.
Figure 1: FXR agonism accelerates PVE-induced liver growth. Animals were pre-treated with the FXR agonist obeticholic acid (OCA) for 7 days before undergoing embolization of the cranial liver lobes. Volume of the total (TLV), caudal (CLV, regenerating after PVE) and cranial lobes (CrLV, atrophying after PVE) was assessed during the course of the experiment. Animals were sacrificed at three and seven days after PVE. A: TLV per kg body weight in the pretreatment phase. Data were analyzed with Wilcoxon matched pairs signed rank and Mann-Whitney U-tests. N=17 per group. B: Increase in CLV following PVE. Values represent percentage increase relative to volume at day -1. Data were analyzed using Mann-Whitney U-tests on area under the curve values at individual time points. N=17 per group until day +3 and N=11 per group at day +7. C: Decrease in CrLV following PVE. Values represent percentage decrease relative to volume at day -1. Data were analyzed using Mann-Whitney U-tests on area under the curve values at individual time points. N=17 per group until day +3 and N=11 per group at day +7. D: Total liver uptake of $^{99m}$Tc-mebrofenin determined by hepatobiliary scintigraphy. Data were analyzed using repeated measurements ANOVA. N=6 per group E: Increase in the share of the caudal liver lobe to $^{99m}$Tc-mebrofenin uptake from baseline measurements at day -7. Data were analyzed using two-way ANOVA. N=5-6 per group. F: Ki67-stained liver sections at 100× magnification and quantification of Ki67-positive hepatocytes. Data were analyzed using Mann-Whitney U-tests. G: Percentage change in body weight relative to values at day -7. Data were analyzed using two-way ANOVA. N = 17 per group. * indicates P < 0.05, ** indicates P < 0.01, and *** indicates P < 0.001 between groups. Abbreviations: OCA, obeticholic acid; TLV, total liver volume; CLV, caudal liver volume; CrLV, cranial liver volume; TLF, total liver function; CLF, caudal liver function.
**Obeticholic acid is not associated with notable hepatocellular and biliary injury**

To examine whether OCA treatment results in liver injury, transaminase levels were measured during the course of the experiment. PVE induced a transient elevation of ALT and AST, with levels peaking at day +1 in both groups and returning to baseline values afterwards (Fig 2A/B). Levels were similar between groups throughout the experiment (Figure 2A, B). yGT and ALP also remained stable in both groups prior to PVE. After PVE yGT and ALP were slightly higher in OCA-treated animals, however levels did not increase above baseline after PVE in either group. (Figure 2C, D). These results show that OCA did not cause hepatocellular injury or cholestasis.

**Figure 2:** Plasma ALT (A), AST (B), yGT (C), and ALP (D) levels before and after PVE. Data are presented as mean (± SEM) for n=5-11 per group. Differences between groups were tested using two-way ANOVA. * indicates P < 0.05, ** indicates P < 0.01. E: Quantitative scoring of H&E-stained sections of the caudal liver lobe. Data are presented as median (range) for n=5-6 per group. Representative liver sections of both groups at day +3 and +7 with H&E staining at 100× magnification. Abbreviations: OCA, obeticholic acid; h, hours; ALT alanine transaminase; AST, aspartate transaminase; yGT, gamma-glutamyl transferase; ALP, alkaline phosphatase.

To examine potential effects of OCA on liver histology, H&E stained sections of the caudal lobe were scored in a blinded fashion. Mild portal and lobular inflammation and mild sinusoidal dilatation were observed in all animals with no differences between groups (Figure 2E). A foreign body reaction caused by backflow of some embolic material in the caudal lobe, was
observed in a total of four animals (two in the control group sacrificed at day +3, one in the OCA group sacrificed at day +3 and one in the OCA group sacrificed at day +7). This was not different between groups and the backflow of embolic material did not appear to affect liver hypertrophy. Small droplet macrovesicular steatosis was observed in both groups at day +3 and +7, with no differences between groups which is consistent with previous observations of mild steatosis in a mouse model of partial hepatectomy.\(^\text{20}\)

**OCA activates ileal and hepatic FXR**

In order to assess transcriptional effects of OCA, FXR target gene expression was analyzed in terminal ileum and liver harvested at 3 and 7 days after PVE. FXR is expressed in both terminal ileum and the liver, and it is conceivable that both contribute to liver regeneration in the rabbit.\(^\text{5,6}\) OCA had no effect on ileal expression of \(\text{Fxr}\) per se, but resulted in induction of ileal \(\text{Shp}\) at day +3 (Figure 3A). This is in line with \(\text{Shp}\) being a target gene of \(\text{Fxr}\). Ileal expression of \(\text{Ost}\), reported to be regulated by \(\text{Fxr}\) in rodents and humans,\(^\text{21,22}\) was however not affected by OCA. We were unable to detect \(\text{Fgf19}\) mRNA in intestinal specimens or liver from control animals (data not shown), despite a study reporting expression in these tissues in the rabbit.\(^\text{23}\) Lack of an empirically validated reference sequence of rabbit \(\text{Fgf19}\) mRNA may underlie this failure. Hepatic expression of the bile salt synthetic enzyme \(\text{Cyp7a1}\) was strongly suppressed in OCA-treated animals at day +3 (Figure 3B). This occurred without transcriptional induction of the repressor \(\text{Shp}\), suggesting that \(\text{Shp}\)-independent \(\text{Fgf19}\) signaling may be responsible for the observed repression of \(\text{Cyp7a1}\). The hepatic receptor for \(\text{Fgf19}\) is formed by \(\text{Fgfr4}\) and \(\beta\)-Klotho. Expression of \(\text{Fgfr4}\) was not affected by OCA treatment, while minor downregulation at the transcript level was observed for \(\beta\)-Klotho after three days (Figure 3B). These findings are consistent with downregulation of \(\text{Cyp7a1}\) by OCA-mediated induction of ileal \(\text{Fgf19}\).

OCA treatment resulted in reduced hepatic \(\text{Fxr}\) expression at day +7. \(\text{Ost}\) expression in the liver was elevated in OCA treated animals at day +3. Conversely, while ileal \(\text{Shp}\) expression was induced by OCA, this was not observed in the liver. The period between last OCA dose and sacrifice ranged between 9-12 hrs, and may have been suboptimal to observe consistent long-term transcriptional effects. Nonetheless, the collective gene expression data indicates that OCA treatment resulted in activation of both ileal and hepatic FXR.
Figure 3: Effect of obeticholic acid on ileal and hepatic gene expression. Animals were pre-treated with obeticholic acid (OCA) for 7 days and underwent embolization of the cranial liver lobes. Terminal ileum (panel A) and caudal liver (panel B) was harvested at three and seven days after PVE. Gene expression was analyzed by RTqPCR. Data are expressed as fold expression compared to the vehicle group. Data are presented as mean (± SEM) for n=5-6 per group. * indicates P < 0.05 and ** indicates P < 0.01 between groups. Abbreviations: OCA, obeticholic acid.

DISCUSSION

In this study the effect of the potent FXR agonist OCA on liver regeneration was examined in a rabbit model of PVE. OCA accelerated liver regeneration in the 7 days after PVE, as evidenced by a 2.2-fold increase in CLV at day +3 and a 1.5-fold increase in CLV at day +7 after PVE in OCA-treated animals compared to vehicle controls. In addition, hepatobiliary scintigraphy revealed an enhanced uptake capacity (+1.2 fold, P=0.02) of the caudal liver lobe 3 days after PVE in OCA-treated animals. This was accompanied by an increase in number of Ki67+ hepatocytes, indicating that PVE plus OCA elicited a stronger hyperplastic response than PVE without OCA. The accelerated liver regeneration induced by OCA holds great clinical potential.
Obeticholic acid accelerates liver regeneration

The current data show that the potent FXR agonist OCA strongly increased the volume gain of the caudal lobe 3 days after PVE in a standardized rabbit model. Increased volume is due to hyperplasia as inferred from enhanced hepatocytic positivity for the proliferative marker Ki67. These results indicate that OCA might be able to reduce the time from PVE to liver resection, which could have several advantages such as avoiding the need for chemotherapy after PVE. Moreover, OCA might improve the liver’s growth response to PVE, which could increase the resectability of patients with very small FLRs. Furthermore, when these results are extrapolated to hepatectomy, the increased liver regeneration by OCA may prevent post-resectional liver failure. Patients are most prone to liver failure the first days after extended liver resection,24 and early initiation of liver regeneration is associated with lower incidences of liver failure.25 Through enhancing liver regeneration in these first days, OCA is expected to reduce the risk of liver failure and resultant morbidity and mortality.

The volumetric as well as functional gain and increased number of Ki67+ hepatocytes indicate accelerated regeneration, which was most pronounced 3 days after PVE. It was previously shown that functional increase of FLR in patients precedes volumetric increase after PVE,26. Thus, OCA-treated rabbits may have increased uptake capacity over controls already in the initial period after PVE, prior to assessment of liver function at day +3. Since liver volume increase is generally slower compared to functional increase,26 the difference in liver volume is still present on day +7. In line with the higher metabolic rate in rabbits, liver regeneration occurs at a higher pace in rabbits compared to humans, whereas in mice and rats metabolism is even higher than in rabbits. The median increase in CLV of 67-71% in vehicle-treated rabbits 7 days after PVE15 is comparable to the 62% increase of FLR volume after a median of 34 days in series of select patients who underwent PVE that included segment 4.13 In the setting of associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), CLV increase 7 days after PVE corresponds with a 74% increase in FLR volume after a median of 9 days following the first stage of ALPPS.13, 27 Therefore 7 days post-surgery in rabbits resemble 4 to 5 weeks of PVE in humans, and 9 days after ALPPS. Within these time intervals, OCA could potentially accelerate the liver regeneration process in humans.

OCA is currently evaluated in phase 3 clinical trials and has been shown to reduce histological features of non-alcoholic steatohepatitis, and to decrease plasma levels of ALP, γGT and ALT in patients with primary biliary cholangitis.28, 29 Substantial safety data are currently available, and therefore clinical translation of OCA for stimulation of liver regeneration is within reach. However, there are some uncertainties regarding its use in the setting of PVE and liver resection. Firstly, seven days of pretreatment is recommended to ensure adequate tissue levels of OCA at the time of PVE or resection.19 Tumors in the liver are at the same
time exposed to the potent agonistic effects of FXR. Several reports suggest that direct FXR stimulation of tumors poses no oncologic concerns,\textsuperscript{30-32} and the elevation of circulating FGF19 levels induced by OCA is not expected to increase growth of tumors expressing FGFR4.\textsuperscript{33, 34} Pharmacological safety studies in mice revealed that 2 years exposure to a dose (25 mg/kg/day) exceeding that used in the current study and in ongoing clinical trials, did not result in OCA-related neoplasms (personal communication from Dr. Luciano Adorini Intercept Pharmaceuticals, based on the toxicology report by WIL Research U.S.) Secondly, the current studies were performed in young healthy rabbits while surgical practice is dominated by elderly patients often with hepatic parenchymal disease,\textsuperscript{35} which both have been shown to decrease the regenerative potential of the liver.\textsuperscript{3} Some reports suggest alleviation of age-related defects in liver regeneration through FXR agonism.\textsuperscript{11} The effects of OCA on regeneration of aged and diseased livers remains to be established. In addition, long term effects as well as OCA dosage and timing variations remain to be evaluated.

In conclusion, this is the first report on OCA as a pharmacological intervention that effectively enhances liver regeneration. Using OCA in a rabbit model of PVE, accelerated liver growth was observed in terms of liver volume, liver function and hepatocyte proliferation, without signs of hepatic injury as assessed by histology and plasma transaminases. OCA has potential in increasing the efficacy of PVE and reducing the time from PVE to liver resection, as well as in the prevention of post-resectional liver failure following major hepatectomy.
REFERENCES


Obeticholic acid accelerates liver regeneration

**Figure S1**: CLV determined by CT volumetry correlated to caudal liver lobe weight at sacrifice. Gray squares represent animals from the control group, black triangles represent animals treated with OCA. Correlation was tested using Spearman's rank correlation coefficient.

**Table S1**: Histologic scoring

<table>
<thead>
<tr>
<th></th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobular inflammation</td>
<td>0 - None 1 - Mild 2 - Moderate 3 - Severe</td>
</tr>
<tr>
<td>Intralobular inflammation</td>
<td>0 - None 1 - &lt;2 foci 2 - 2 to 5 foci 3 - &gt; 5 foci</td>
</tr>
<tr>
<td>Sinuoidal dilatation</td>
<td>0 - None 1 - Mild 2 - Moderate 3 - Severe</td>
</tr>
<tr>
<td>Foreign body reaction in portal veins</td>
<td>0 - No 1 - Yes</td>
</tr>
<tr>
<td>Small droplet microvesicular steatosis</td>
<td>0 - None 1 - Mild 2 - Moderate 3 - Severe</td>
</tr>
</tbody>
</table>

**Table S2**: Primer sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rplp0</td>
<td>CCTCGTGAGAGTGACATCGT</td>
<td>CGCCACGATGAAAGCATTTT</td>
</tr>
<tr>
<td>Hprt</td>
<td>GACCACTCAACAGGGGACAT</td>
<td>ATCCAAACTGCTGCGCTGTG</td>
</tr>
<tr>
<td>Gapdh</td>
<td>CCACACTCATGGTCTACATGGTCC</td>
<td>TCACCCACTGATTGGGCC</td>
</tr>
<tr>
<td>Fxr</td>
<td>ACAAGTACAGCTGACAAAGCA</td>
<td>AGGCTAGCCACCCCTTGCAAC</td>
</tr>
<tr>
<td>S1c51b</td>
<td>TGGGAACAGAGGAGCCAGAAAC</td>
<td>GTCAAGGGAAGGATGAAAT</td>
</tr>
<tr>
<td>Sph</td>
<td>GCCCAAGGAAATACCGCTAC</td>
<td>CGGATAAGACTGAGGGGTG</td>
</tr>
<tr>
<td>Fgfr4</td>
<td>GAAACACAGCAATGGCCCG</td>
<td>GACCGCCCAAGTGAGAGAT</td>
</tr>
<tr>
<td>Klb</td>
<td>GAGAAGGCGCTGTCCTGACAGA</td>
<td>TCGAGCACCTGAGGAGAAGA</td>
</tr>
<tr>
<td>Cyp7a1</td>
<td>ATATGATGAGGAGCTGAGGC</td>
<td>GGGACTCCTGATGAGTCTGT</td>
</tr>
</tbody>
</table>
Figure S2: Hepatobiliary scintigraphic images of a single rabbit at all sequential scans. The yellow region of interest (ROI) delineates the left ventricle, the red ROI the total liver and the pink ROI the caudal liver lobe. A marked decrease in cranial liver lobe activity and increase in caudal liver lobe activity was seen following portal vein embolization.