Effect of ursodeoxycholic acid on the impairment induced by maternal cholestasis in the rat placenta-maternal liver tandem excretory pathway

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Abbreviations:
BDL, bile duct ligation; GC, glycocholate; mTPM, maternal-facing trophoblast plasma membrane; Mrp, Multidrug resistance associated protein; Oatp, Organic anion-transporting polypeptide; OCP, obstructive cholestasis during pregnancy; rPL II, rat placental lactogen type II; UDCA, ursodeoxycholic acid.

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ABSTRACT

We investigated the effects of ursodeoxycholic acid (UDCA, 60 µg/day/100 g b.wt.) on the impairment induced by maternal obstructive cholestasis during pregnancy (OCP) in the rat placenta-maternal liver tandem excretory pathway. A blunted catheter was implanted in the common bile duct on day 14 of pregnancy and the tip was cut on day 21. [14C]-Glycocholate (GC) was then administered through the umbilical artery of “in situ” perfused placenta (placental transfer test) or through the maternal jugular vein (biliary secretion test) and GC bile output was measured. OCP impaired both GC placental transfer and maternal biliary secretion. UDCA moderately improved the latter but had a more marked beneficial effect on GC placental transfer. Histological examination revealed trophoblast atrophy and structural alterations, e.g. loss of apical membrane microvilli in OCP placentas. Gene expression level was investigated by real-time quantitative RT-PCR and western blot analysis. OCP reduced both placental lactogen-II (a trophoblast-specific gene) mRNA and the functional amount of epithelial tissue, determined by transplacental diffusion of antipyrin. Using a rapid filtration technique, impairment in the ATP-dependent GC transport across trophoblast apical plasma membranes obtained from OCP placentas was found. UDCA partially prevented all these changes. The expression level of organic anion transporters: Oatp1, Oatp2 and Oatp4; and multidrug resistance-associated proteins: Mrp1, Mrp2 and Mrp3 in whole placenta were not or moderately affected by OCP but greatly enhanced by UDCA. In sum, UDCA partially prevents deleterious effects of OCP on the rat placenta-maternal liver tandem excretory pathway, mainly by preserving trophoblast structure and function.
INTRODUCTION

Carrier proteins located in the basolateral and apical plasma membranes of hepatocytes are responsible for bile acids (BAs) uptake and secretion into bile (Meier and Stieger, 2002). During intrauterine life, when the liver is not yet mature, efficient transfer of fetal BAs across the placenta, together with normal maternal hepatobiliary function maintain fetal BA levels within the physiological range. Previous studies have shown that carriers located in the trophoblast plasma membranes play a role in the vectorial fetal-to-maternal translocation of cholephilic compounds, such as BAs (Marin et al., 1990; Marin et al., 1995) and bilirubin (Serrano et al., 2002). Maternal hypercholanemia may challenge transplacental elimination of fetal BAs through the creation of inversely directed gradients as compared to the physiological situation (Monte et al., 1995), and by impairing the placental ability to carry out vectorial BA transfer (Macias et al., 2000). Moreover, obstructive cholestasis during pregnancy (OCP) in rats results in an accumulation of BAs in fetuses, a situation that is also transiently observed later in juvenile animals born from cholestatic mothers (Monte et al., 1996). Exposure of the fetal liver to high BA levels is probably involved in the delayed maturation of hepatobiliary function observed in these young rats (Monte et al., 1996; El-Mir et al., 1997).

In humans, intrahepatic cholestasis of pregnancy (ICP) is accompanied, among other maternal and fetal alterations (Reid et al., 1976; Mullally and Hansen, 2002), by a reduced ability to transport BAs across the trophoblast plasma membrane (Serrano et al., 1998). Treatment with ursodeoxycholic acid (UDCA), which has beneficial effects in several cholestatic liver diseases (Poupon and Poupon, 1995; Lazaridis et al., 2001; Kowdley, 2000), is able to mitigate pruritus and enzyme elevations in the serum of women with ICP (Palma et al., 1992; Floreani et al., 1994; Brites et al., 1998). Because UDCA was also found to have a positive effect on ICP placentas, at least as far as BA transport by trophoblast plasma membrane vesicles was concerned (Serrano et al., 1998), in the present study we intended to further characterize structural and functional aspects of the beneficial effects of UDCA on maternal hypercholanemia-induced impairment of the rat placenta-maternal liver tandem excretory pathway for fetal BAs.
MATERIALS AND METHODS

Experimental groups

Non-fasting pregnant Wistar CF rats (Animal House, University of Salamanca, Spain) were used. The experiments were approved by the Local Ethical Committee. On day 14 of pregnancy all animals were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p., Abbot, Madrid, Spain) to either sham-operate them (Control) or produce a complete obstructive cholestasis (OCP) by implantation of a blunted catheter in the common bile duct (Monte et al., 1996). During the following week part of these animals received daily intragastric administration of 60 µg UDCA in 60 µL of 75 mM NaCl, 50 mM Na₂CO₃, pH 8.3, per 100 g b.wt. (OCP+UDCA group, n=60). The rest of the cholestatic rats (OCP group, n=70) received only the vehicle.

“In vivo” experiments

On day 21 the animals were anesthetized again. Cannulation of the common bile duct was performed in Controls. In OCP and OCP+UDCA groups the blunted tips of the catheters implanted were cut, thereby allowing free bile flow. This was measured gravimetrically. BA concentrations were determined enzymatically (Talalay, 1960). The doses of [¹⁴C]-glycocholate ([¹⁴C]-GC; specific radioactivity 46.7 mCi/mmol) used in different types of experiments were selected based on previous studies and were administered once BA output had reached an approximate steady-state after bile drainage (Macias et al., 2000). To evaluate BA uptake and secretion by the maternal liver 5 nmol [¹⁴C]-GC in 150 mM NaCl, 5 mM glucose was administered through the maternal jugular vein as a 5 min bolus (50 µL/min). Transfer of [¹⁴C]-GC across the placenta-maternal liver tandem was measured using "in situ" single-pass perfused placenta. 250 nmol [¹⁴C]-GC was administered with the perfusate - 137 mM NaCl, 2.7 mM KCl, 1.05 mM MgCl₂, 1.80 mM CaCl₂, 12 mM NaHCO₃, 0.4 mM NaH₂PO₄, 5 mM glucose, 0.05% (w/v) heparin, 10 mM Hepes, pH 7.40 at 37°C - through the umbilical artery over 5 min (500 µL/min) and radioactivity in maternal serum and bile was determined (Macias et al., 2000, Briz et al., 1998). Placental [¹⁴C]-GC content was measured in similar experiments, except that 2 or 10 min after finishing [¹⁴C]-GC administration the perfused placenta and the remaining non-perfused placentas and their fetuses were collected to measure radioactivity. The amount of tissue able to carry out placental exchange was determined by measuring the magnitude of the diffusional pathway for antipyrine. Fetal/maternal serum antipyrine concentration ratios were assayed as previously described.
Experiments on plasma membrane vesicles

Maternal-facing trophoblast plasma membrane (mTPM) vesicles were isolated from rat placenta as previously described (Macias et al., 2000, Bravo et al., 1998). Enrichment of mTPM associated marker alkaline phosphatase activity over the homogenate did not differ significantly between Control (12.0±3.1–fold, n=5), OCP (16.2±2.2-fold, n=5) and OCP+UDCA (15.8±1.3-fold, n=5) groups. Low contamination with fetal-facing trophoblastic basal plasma membrane fragments was indicated by similar de-enrichments of dihydroalprenolol binding in Control (0.5±0.2-fold, n=5), OCP (0.4±0.1-fold, n=5) and OCP+UDCA (0.5 ±0.1-fold, n=5) groups. Membrane vesicles were resupended in buffer A (250 mM sucrose, 0.2 mM CaCl$_2$, 10 mM MgCl$_2$, 100 mM KNO$_3$, 10 mM Hepes/Tris, pH 7.40) and stored in liquid nitrogen. Before carrying out the experiments, membranes were first thawed, then diluted with buffer A to approximately 5 mg protein/ml and vesiculated by six passages through a 25-gauge needle. Protein was determined (Markwell et al., 1978) using bovine serum albumin as standard. Using a rapid filtration technique (Marin et al., 1990, Macias et al., 2000) ATP-dependent $[^{14}\text{C}]-\text{GC}$ uptake by mTPM vesicles was measured during the initial linear uptake phase (30 sec) at 37°C.

Determination of gene expression

RNA from snap-frozen placentas and livers was isolated using RNeasy spin columns from Qiagen (Izasa, Barcelona, Spain) and measured with the RiboGreen RNA-Quantitation kit (Molecular Probes, Leiden, The Netherlands). Reverse transcription was carried out with 1 µg of total RNA, using random nanomers and Enhanced Avian RT-PCR kit (Sigma-Genosys, Cambridge, UK).

The presence of the selected mRNAs was investigated by the detection and subsequent sequencing of mid-size fragments (Table 1) of specific cDNA amplified by 45 cycles of hot-start PCR (AmpliTaq Gold Polymerase, Applied Biosystems, Madrid, Spain). Real-time quantitative PCR was then performed (ABIPrism-5700, Perkin-Elmer Applied Biosystems), using conditions shown in Table 1. The PCR amplification products were detected using SYBR Green I, once it had been ascertained that nonspecific products had been formed in PCR reactions in all cases. Total liver RNA from a healthy male adult rat (for Oatps and
Mrp2) or from a male adult rat with bile duct ligation (BDL) for 7 days (for Mrp1 and Mrp3) were used in all determinations as external calibrators and the levels of 18S rRNA in each sample was used to normalize the results.

Because major changes affected mRNA levels of a typical basolateral carrier (i.e., Oatp2) and a typical apical exporter (i.e., Mrp2), for which antibodies were available, they were selected to carry out western blot analysis on crude placental cell membrane preparations. As positive controls we used rat liver basolateral (bLPM) and canalicular (cLPM) plasma membranes (Bossard et al., 1993). Membrane preparations (15 µg protein of either bLPM or cLPM, or 50 µg protein of placental cell membranes) were diluted in sample buffer under reducing conditions, incubated for 3 min at 100°C, subjected to 7.5% or 15% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Polyclonal antibodies against rat Oatp2 (K15, dilution 1:7500) and Mrp2 (K13, dilution 1:3000) were used as primary antibodies.

**Histological examination and data analysis**

Light and transmission electron microscopical examinations of placental tissue were carried out as previously reported (Macias et al., 2000). Morphometric analysis was performed using NIH Image V1.62 software (National Institutes of Health, USA).

Results are means ± SEM. Significance of differences of OCP and OCP+UDCA versus Control was determined by the Bonferroni method of multiple range testing. Kinetic parameters were calculated after fitting the data from transport studies with an iterative non-linear least-squares method, using the UltraFit-v2.1 software (Biosoft, Cambridge, UK) to the Michaelis-Menten equation for a single carrier system. To characterize pharmacokinetic parameters a model-independent method based on the theory of statistical moments was used (Yamaoka et al., 1978).
RESULTS

Morphological, biochemical, histological and molecular changes

Morphological and biochemical changes are in agreement with previous studies using OCP rats (El-Mir et al., 1997; Macias et al., 2000; Monte et al., 1996). Some of them were prevented by UDCA (Table 2). Atrophy of trophoblastic tissue in OCP placentas with dilated maternal vascular lacunae - to approximately 189% of Control - was seen. These alterations were in part corrected in OCP+UDCA placentas in which morphometric analysis revealed that fetal tissue was restored to 92% of Control value (Figure 1). At subcellular level (Figure 2) syncytiotrophoblastic cells from the OCP group displayed hydroponic degeneration. Cells appeared with a very dilated pericanalicular space, a highly vacuolar cytoplasm, and a lack of microvilli on mTPM. These alterations were partly prevented by UDCA. The proportion of trophoblast in the placentas, as determined by placental lactogen type II (rPLII) - a specific trophoblastic marker during the last stages of rat gestation (Faria et al., 1990; Shah et al., 1998) - was reduced by OCP, but restored by UDCA (Figure 3B).

When mRNA level was related to that of rPLII, increases in relative abundance of mRNA for all studied carriers in OCP and OCP+UDCA were observed (Figure 3C). When total placental level was calculated, no change or a tendency towards increased expression of BA and organic anion carriers in whole OCP placentas, that was statistically significant only for Oatp1 and Oatp2, was found. However in OCP+UDCA placentas the mRNA levels for all of them were enhanced (Figure 3B). The highest levels were for Oatp2 and Mrp2. Western blot analysis of these two representative carriers, indicated that they were not detectable by this technique in Control (Figure 3B). However, consistently with changes in mRNA levels, they were seen in OCP and OCP+UDCA placentas as clear bands, but not intense enough to permit accurate quantitative analysis (Figure 3A).

“In vitro” functional studies

Kinetic parameters for ATP-dependent \[^{14}\text{C}]\text{-GC} uptake by mTPM versus substrate concentrations (Figure 4A) indicated that, in agreement with previous studies (Macias et al., 2000), the transport efficiency, as defined by the Vmax-to-Km ratio, was reduced in mTPM from OCP placentas. This was mainly caused by an increase of the Km. UDCA treatment did not significantly modify Vmax but restored the Km-value to normal and hence reversed the efficiency of this transport to values close to those of Control placentas (Figure 4B).
“In vivo” functional studies

Once bile flow is allowed on day 21, this and BA output were initially higher in OCP and OCP+UDCA than in Control (Figure 5). This was followed by a progressive decrease in BA output below Control levels, probably due to the fact that, in the absence of a normal enterohepatic circulation, most of the BA pool is located in the liver. By contrast, after an initial decrease, bile flow was maintained in both OCP and OCP+UDCA at a similar level to that found in Control. Cholangiocyte proliferation and changes in its secretory function associated to rat biliary obstruction for 1 week (Alpini et al., 2002) might well account for an increased contribution of ductular components to bile formation.

Antipyrine administration through the maternal jugular vein resulted in similar steady-state values in maternal serum antipyrine concentrations in all groups after 9 min (data not shown). Steady-state, also reached in fetal serum antipyrine concentrations at this time in Controls, was characterized by values of fetal-to-maternal serum antipyrine concentration ratio close to 1.0 (Figure 6). Steady-state was reached in both OCP and OCP+UDCA 3 min later, and the values for the fetal-to-maternal ratio were lower than 1.0 (OCP: 0.75; OCP+UDCA: 0.83).

When $[^{14}\text{C}]$-GC was administered through the umbilical artery maximal $[^{14}\text{C}]$-GC output (14 pmol/min/g liver) was attained at 15 min in Control group (Figure 7). This was lower (2 pmol/min/g liver) and was reached later (75 min) in OCP rats. UDCA partially restored $[^{14}\text{C}]$-GC placental BA transfer. From results shown in Table 3 it can be calculated that, in agreement with previous studies (Macias et al., 2000), the fraction of the $[^{14}\text{C}]$-GC dose administered through the umbilical artery that was secreted into bile over 2 h after administration was 2.15% in Controls. This was greatly reduced to 0.54% by OCP and partly restored to 1.15% in OCP+UDCA group. No detectable radioactivity was found in unperfused placentas or their fetuses collected at 2 or 10 min, (data not shown). When tissue collection from perfused placentas was carried out 2 min after the end of $[^{14}\text{C}]$-GC administration, the dose fraction of $[^{14}\text{C}]$-GC taken up into placentas (administered dose = 100%) calculated from results shown in Table 3 was Control (2.22%) > OCP+UDCA (1.21%) > OCP (1.01%).

A comparison of the amount of $[^{14}\text{C}]$-GC taken up into placentas and the cumulative $[^{14}\text{C}]$-GC output into maternal bile for 120 min revealed that approximately the same amount as that initially detected in the perfused placenta was secreted by the maternal liver over this time in
both the Control and OCP+UDCA groups, but not in the OCP animals, in which the amount secreted into bile was significantly lower than that taken up by the placenta (Table 3). Measurements of radioactivity in placentas at min 10 as compared with that found at min 2 indicated that the proportion of $[^{14}C]$-GC remaining in this organ after that time was smaller in Controls than in the other two groups (Table 3).

When $[^{14}C]$-GC was injected through the maternal jugular vein (Figure 8) $[^{14}C]$-GC was rapidly secreted into bile, peaking at approximately 10 min in the Control group. In these rats, the amount of $[^{14}C]$-GC collected during the first 60 min after administration was approximately 100% of the dose injected. A delayed $[^{14}C]$-GC output into bile was observed in OCP, which was not corrected by UDCA (Table 3). Thus, there was a 5-min delay in the appearance of the secretion peak and $T_{1/2}$ was prolonged in OCP OCP+UDCA. However, cumulative $[^{14}C]$-GC secretion (AUC) over 2 h after administration was not significantly different in Control and OCP+UDCA groups, while it was moderately reduced in the OCP group (Table 3).
DISCUSSION

In agreement with previous findings by our group and others (Macias et al., 2000, Klaassen, 1974), diminished and slower secretion of exogenously administered bile acids to rats after releasing obstructive cholestasis was observed. This is consistent with the known reduction occurring after BDL in the expression of rat sinusoidal BA carriers, while canalicular exporter pumps, Mrp2 and Bsep are decreased or partially maintained, respectively (Trauner et al., 1999).

Although improvement of the drug-induced cholestasis in the rat by UDCA has been reported (Jacquemin et al., 1993), its beneficial effect after BDL is limited (Poo et al., 1992; Purucker et al., 2001; Hinz et al., 1997). In the present study, UDCA administration to OCP rats also resulted in only a partial beneficial effect on liver ability to secrete \([^{14}C]\)-GC. Nevertheless, this probably contributed to enhance the elimination of \([^{14}C]\)-GC when this was administered through the umbilical artery.

One of the most striking findings of the present study was the effect of UDCA on trophoblast. Morphological, molecular and functional measurements indicated a dramatic reduction in the amount of functional trophoblast in OCP placentas. As has been reported by others in several tissues (Abdel-Aziz et al., 1991), inflammatory processes associated with BA accumulation probably account for fibrogenesis, accumulation of extracellular matrix and necrosis also present in OCP placentas (data not shown), all of which may contribute to reducing transplacental exchange. UDCA prevented the OCP-induced loss of trophoblast, structural alterations were limited and the functional test based on antipyrine diffusion was partly restored.

Another important contribution is the confirmation of preliminary observations (St-Pierre et al., 1999) regarding the presence of Oatp1, Oatp2 and Oatp4 in rat placenta. Assuming that these carriers are located only in the trophoblast, it could be calculated that the relative abundance of their mRNA as compared to rPL II was enhanced in OCP and OCP+UDCA. However, because the amount of trophoblast was reduced by OCP, the total levels in whole placentas of this group were not markedly higher than in Control. By contrast, because of UDCA restored the amount of trophoblast, total placental mRNA levels for these carriers were enhanced in a moderate-to-marked range in OCP+UDCA. This could in part account for
the partial recovery of the efficiency of placental $^{14}\text{C}$-GC uptake in this group. The fact that $^{14}\text{C}$-GC uptake was markedly reduced in OCP, suggests that “adaptative” changes in the level of mRNA for these carriers in the remaining trophoblast were insufficient to compensate the negative effects due to OCP-induced structural and functional alterations.

While placental $^{14}\text{C}$-GC uptake occurred over 5 min, its output toward the mother and then into bile took much longer, especially in the OCP animals, supporting the concept that an export mechanism located in the apical membrane of the trophoblast is probably the limiting step in the overall process of BA elimination by the placenta-maternal liver tandem (Briz et al., 1998).

The marked OCP-induced impairment in $^{14}\text{C}$-GC transfer across the apical membrane of the trophoblast, was consistent with lower efficiency of ATP-dependent transport found in studies with plasma membrane vesicles. This transport is probably mediated by one or several members of the superfamily of ATP-binding cassette (ABC) proteins. Some of the human MRP orthologs have been identified in human placenta (St-Pierre et al., 2000). Similarly to what happens in the liver (except for Mrp2) and kidney (Lee et al., 2001; Pei et al., 2002; Soroka et al., 2001) the adaptive up-regulation of several Mrp transporters in the rat trophoblast was stimulated by OCP.

Increases in the levels of mRNA for uptake and export carriers were probably accompanied by enhancements in the amount of these proteins in trophoblastic cells, as indicated by western blot analysis of two representative carriers, Oatp2 and Mrp2. However, the lack of normal micrivilli in trophoblast plasma membranes in OCP placentas together with the fact that, even when purified apical membranes were used, the efficiency of ATP-dependent $^{14}\text{C}$-GC transport was reduced in this group, suggests that carrier overexpression was not enough to counterbalance other negative changes in the plasma membrane composition/structure or in the maturation/sorting process affecting the function of the carriers and that such negative effects would be responsible for the reduction in carrier ability to export BAs.

Moreover, the possibility that part of the discrepancy between enhanced expression levels and reduced transport ability could be attributed to the fact that some of these transporters may be present in intracellular compartments, which are not actively involved in BA transport across the trophoblast, should be considered. Treatment with UDCA did not reduce the relative
expression of Mrps in the trophoblast, which together with the UDCA-induced recovery of the amount and structure of this tissue contributed to restoring $[^{14}\text{C}]-\text{GC}$ transfer into the maternal compartment.

The overall UDCA-induced beneficial effects had important consequences on the conceptus development. Thus the intrauterine growth retardation observed in OCP fetuses was prevented by UDCA. It should be kept in mind that OCP resulted in a diminished presence of BAs in the maternal gut that was corrected by gavage supplementation with UDCA. This may determine better intestinal lipid digestion and liposoluble vitamin absorption (Mullally and Hansen, 2002), which together with displacement of more toxic BA species in the maternal-fetal BA pool and the beneficial effect on the placenta and maternal liver functions, all probably play an important role in normal fetal growth and the number of fetuses per pregnancy found in the OCP+UDCA group. This is consistent with the clinical results of UDCA therapy in ICP, where the compound reduces the number of stillbirths and perinatal mortality (Palma et al., 1997; Davies et al., 1995).
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REFERENCES


Footnotes

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FIGURE LEGENDS

Figure 1. Light microscopy images from hematoxylin-eosin staining of tissue slices obtained from placentas collected from 21-day pregnant rats. Samples were from animals representative of each of the experimental groups, which were Controls and rats undergoing surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP) or 60 µg UDCA/100g b.w./day (OCP+UDCA). C, Conective tissue; CT, cytotrophoblastic cells; F, fetal vascular spaces containing fetal blood red cells; M, maternal vascular spaces; ST, syncytiotrophoblastic cells.

Figure 2. Transmission electron microscopy images from tissue slices obtained from placentas collected from 21-day pregnant rats. Samples were from animals representative of each of the experimental groups, which were Controls and rats undergoing surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP) or 60 µg UDCA/100g b.w./day (OCP+UDCA). Original magnification x 4000.

Figure 3. Expression level of the trophoblast-specific gene rat placental lactogen-II (rPL-II), the organic anion transporters: Oatp1, Oatp2 and Oatp4; and the multidrug resistance-associated proteins: Mrp1, Mrp2 and Mrp3, in placentas at day 21 of pregnancy. A. Representative western blot analysis for Oatp2 and Mrp2 in placental cell membranes from pregnant rats of each experimental group, i.e., Controls, OCP (surgical common bile duct obstruction on day 14 of pregnancy) and OCP+UDCA (biliary obstruction on day 14, followed by treatment with 60 µg UDCA/day/100g b.w. for the remaining 7 days). As positive control, male rat liver basolateral (bLPM) and canalicular (cLPM) were included. B. Relative abundance of mRNA determined using the real-time quantitative RT-PCR and expressed as percentages of the Control group. C. Relative abundance of mRNA corrected by the expression level of rPLII, which was considered as representative of the amount of trophoblast tissue. Values (means±S.D.) were from total RNA isolated from placentas belonging to Control (n=5), OCP (n=5) and OCP+UDCA (n=5) groups. *, p<0.05, on comparing OCP and OCP+UDCA with Control by the Bonferroni method of multiple range testing.
Figure 4. A. ATP-dependent $^{14}$C-glycocholate ($^{14}$C-GC) uptake by apical plasma membrane vesicles obtained from placentas collected from 21-day pregnant rats. Experimental groups were Controls (n= 8) and rats undergoing surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP group, n=8) or 60 µg UDCA/100g b.w./day (OCP+UDCA group, n=7). Values were obtained from experiments carried out in triplicate for each substrate concentration. ATP-dependent uptake was calculated by subtracting uptake values in the absence of ATP from uptake values in the presence of ATP. B. Kinetic parameters calculated from fitting the results to a Michaelis-Menten equation for a single carrier system: $V_0 = (V_{\text{max}} \times S) / (K_M + S)$; where $V_0$ is the initial velocity of $^{14}$C-GC uptake; $S$ is substrate concentration in the incubation medium; $K_M$ is the apparent affinity constant or Michaelis-Menten constant, and $V_{\text{max}}$ is the maximal transport velocity. Values are means±S.D. *, p<0.05 on comparing OCP and OCP+UDCA with Controls by the Bonferroni method of multiple range testing.

Figure 5. Time-course of bile flow (A) and bile acid output (B) in anesthetized pregnant rats at term (day 21) for 180 min bile collection through a catheter implanted into the common bile duct in Control rats (closed squares, n=21) and in rats that underwent surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP group, open circles, n=17) or 60 µg UDCA/100g b.w./day during the last week of gestation (OCP+UDCA group, closed circles, n=11). Values are means±SEM. *, p<0.05 on comparing Controls with OCP group; †, p<0.05 on comparing Controls with OCP+UDCA group, by the Bonferroni method of multiple range testing.

Figure 6. Fetal-to-maternal ratios of serum antipyrine concentrations during its administration through the jugular vein (100 mg bolus followed by 0.5 mg/min continuous infusion) to 21-day pregnant rats. Experimental groups were Controls (closed squares, n= 8) and rats undergoing surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP group, open circles, n=5) or 60 µg UDCA/100g b.w./day (OCP+UDCA group, closed circles, n=4). After bile drainage for 180 min, antipyrine administration started immediately after ligation of the common bile duct in all groups. The inset depicts a schematic representation of the experiment. Values are means±S.E.M. *, p<0.05 on comparing Controls with OCP group; †, p<0.05 on comparing Controls with OCP+UDCA group, by the Bonferroni method of multiple range testing.
Figure 7. Bile output of $^{14}$C-glycocholate ($^{14}$C-GC) following administration (250 nmol over 5 min) through the umbilical artery of one of the "in situ" perfused rat placentas in 21-day pregnant rats. Experimental groups were Controls (closed squares, n= 8) and rats undergoing surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP group, open circles, n=8) or 60 µg UDCA/100g b.w./day (OCP+UDCA group, closed circles, n=6). The inset depicts a schematic representation of the experiment. Values are means±S.E.M. *, p<0.05 on comparing Controls with OCP group; †, p<0.05 on comparing Controls with OCP+UDCA group, by the Bonferroni method of multiple range testing.

Figure 8. Bile output of $^{14}$C-glycocholate ($^{14}$C-GC) following administration (5 nmol over 5 min) through the jugular vein of 21-day pregnant rats. Experimental groups were Control (closed squares, n= 8) and rats undergoing surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP group, open circles, n=8) or 60 µg UDCA/100g b.w./day (OCP+UDCA group, closed circles, n=6). The inset depicts a schematic representation of the experiment. Values are means±S.E.M. *, p<0.05 on comparing Controls with OCP group; †, p<0.05 on comparing Controls with OCP+UDCA group, by the Bonferroni method of multiple range testing.
Table 1. Gene- and species-specific primers used for PCR analysis

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<td>GTGAAGCTTTTCGCTGTAAGGA</td>
<td>TACATGATTGACAGGAAAGGA</td>
<td>AJ 277881</td>
<td>90 bp</td>
<td>742-831</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>CTTGCGTACAAGCTGAGCATAT</td>
<td>AGGCTCTGCTTGGCTCTATAAA</td>
<td>735 bp</td>
<td>2115-2849</td>
<td></td>
</tr>
<tr>
<td>Mrp2</td>
<td>Q</td>
<td>TGATCGTCTTTCGCTGAGAGCT</td>
<td>ACGCACATTCCAACACAAAA</td>
<td>NM_012833</td>
<td>139 bp</td>
<td>1119-1257</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>AAGCGCGAGGGAGACATTAT</td>
<td>ACGCACATTCCAACACAAAA</td>
<td>1181 bp</td>
<td>32-1212</td>
<td></td>
</tr>
<tr>
<td>Mrp3</td>
<td>Q</td>
<td>TCCACTTCTCGAGACAGTAAC</td>
<td>CACCTTGACATCATGAGACCTTT</td>
<td>NM_0080581</td>
<td>90 bp</td>
<td>3481-3570</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>CAGCGACAACGGGTGAGTTT</td>
<td>TCGGTGTCTTGTCAGGTCTGTGT</td>
<td>404 bp</td>
<td>2308-2711</td>
<td></td>
</tr>
<tr>
<td>Oatp1</td>
<td>Q</td>
<td>CTACTGGCCCTTCAAGGCC</td>
<td>ATTGTATCTCTCAAGGATTCCGAGG</td>
<td>L19031</td>
<td>68 bp</td>
<td>2108-2175</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>GCAGAAAGCTGGAAAACCAT</td>
<td>GGGGATGCTGGGCTGAGGATAT</td>
<td>818 bp</td>
<td>55-872</td>
<td></td>
</tr>
<tr>
<td>Oatp2</td>
<td>Q</td>
<td>TGCGGAGATGAGCTTACC</td>
<td>TCCTCGTCATTCCGACCTTT</td>
<td>U88036</td>
<td>69 bp</td>
<td>2032-2100</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>CCAGCTTTCCCTTTTT</td>
<td>AATGCCTGCAGGAGATATA</td>
<td>812 bp</td>
<td>891-1702</td>
<td></td>
</tr>
<tr>
<td>Oatp4</td>
<td>Q</td>
<td>AGACGTCCCCATCACAACCAC</td>
<td>GCCTCGAGTTTCCCTTGA</td>
<td>AJ 271682</td>
<td>68 bp</td>
<td>86-153</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>GCCAACGTCTCCGATCAA</td>
<td>GCCAAGGATGGGTCCAAATCAT</td>
<td>624 bp</td>
<td>152-775</td>
<td></td>
</tr>
</tbody>
</table>

PCR-Q, quantitative polymerase chain reaction; PCR-S, Sequencing polymerase chain reaction. Total liver RNA from a healthy male adult rat (for Oatps and Mrp2) or from a male adult rat with bile duct ligation (BDL) for 7 days (for Mrp1 and Mrp3) were used in all determinations as external calibrators among different sets of reactions. Rat genes: rPLII (rat placental lactogen II), Mrp1 (Abcc1); Mrp2 (Abcc2); Mrp3 (Abcc3), Oatp1 (Slc21a1); Oatp2 (Slc21a5); Oatp4 (Slc21a10).
Table 2. Morphological and biochemical parameters on day 21 of pregnancy

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>OCP</th>
<th>OCP+UDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphological Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Body Weight (g)</td>
<td>385±10</td>
<td>300±10c</td>
<td>326±13a</td>
</tr>
<tr>
<td>Fetal Body Weight (g)</td>
<td>4.9±0.2</td>
<td>3.7±0.2c</td>
<td>4.1±0.2a</td>
</tr>
<tr>
<td>Maternal Liver Weight (g)</td>
<td>11.4±0.4</td>
<td>11.7±0.5</td>
<td>12.3±0.5</td>
</tr>
<tr>
<td>Placenta Weight (g)</td>
<td>0.36±0.02</td>
<td>0.33±0.03</td>
<td>0.34±0.05</td>
</tr>
<tr>
<td>Number of Fetuses</td>
<td>13.0±0.4</td>
<td>10.2±0.8a</td>
<td>12.7±0.9</td>
</tr>
<tr>
<td><strong>Biochemical Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maternal Serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Bile Acid (µM)</td>
<td>15.0±2.1</td>
<td>235.1±25.6c</td>
<td>278±27.3a</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>0.16±0.01</td>
<td>2.91±0.64c</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>GOT (UI/L)</td>
<td>97±13</td>
<td>392±45c</td>
<td>537±61a</td>
</tr>
<tr>
<td>GPT (UI/L)</td>
<td>18.5±1.4</td>
<td>35.8±6.5a</td>
<td>51.1±10.3a</td>
</tr>
<tr>
<td>GGT (UI/L)</td>
<td>5.6±0.8</td>
<td>9.7±1.6a</td>
<td>14.6±2.0a</td>
</tr>
<tr>
<td>Alkaline Phosphatase (UI/L)</td>
<td>94±7</td>
<td>181±21b</td>
<td>130±11a</td>
</tr>
<tr>
<td>LDH (UI/L)</td>
<td>1181±195</td>
<td>1905±137b</td>
<td>2281±302a</td>
</tr>
<tr>
<td><strong>Fetal Serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Bile Acid (µM)</td>
<td>17.4±2.8</td>
<td>46.5±5.6c</td>
<td>32.6±4.5c</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>0.28±0.05</td>
<td>0.61±0.11a</td>
<td>0.33±0.08</td>
</tr>
<tr>
<td>GOT (UI/L)</td>
<td>325±22</td>
<td>350±23</td>
<td>374±25</td>
</tr>
<tr>
<td>GPT (UI/L)</td>
<td>21.8±4.0</td>
<td>26.9±5.1</td>
<td>21.5±3.8</td>
</tr>
<tr>
<td>GGT (UI/L)</td>
<td>6.3±0.5</td>
<td>7.5±0.7</td>
<td>7.1±1.0</td>
</tr>
<tr>
<td>Alkaline Phosphatase (UI/L)</td>
<td>987±62</td>
<td>1231±68a</td>
<td>1323±84a</td>
</tr>
<tr>
<td>LDH (UI/L)</td>
<td>3270±208</td>
<td>2611±379</td>
<td>2575±268</td>
</tr>
</tbody>
</table>

Biochemical parameters were determined in blood samples collected from mothers and fetuses on day 21 of pregnancy, before any other manipulation was carried out. Experimental groups were sham-operated rats (Control, n=18), rats with obstructive cholestasis during the last third of pregnancy (OCP, n=18) and OCP rats treated with UDCA (i.e., 60 µg/100g b.w./day) from day 14 to day 21 of pregnancy (OCP+UDCA, n=15). Values are means±S.E.M. . a, p<0.05; b, p<0.01; c, p<0.001 on comparing OCP and OCP+UDCA to Controls by the Bonferroni method of multiple range testing.
Table 3. Glycocholate placental uptake and bile secretion by maternal liver

<table>
<thead>
<tr>
<th>[¹⁴C]-GC infusion through</th>
<th>CONTROL</th>
<th>OCP</th>
<th>OCP+UDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UMBILICAL ARTERY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental content at min 2</td>
<td>5.56±0.08</td>
<td>2.20±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.02±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>nmol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental content at min 10</td>
<td>1.86±0.12</td>
<td>1.38±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.90±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>nmol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent of uptake</td>
<td>33.4%</td>
<td>62.7%</td>
<td>62.9%</td>
</tr>
<tr>
<td>Cumulative Bile output</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol</td>
<td>5.37±0.15</td>
<td>1.35±0.14&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>2.87±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percent of uptake</td>
<td>96.6%</td>
<td>61.4%</td>
<td>95.0%</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>20.1±1.0</td>
<td>56.2±2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.6±1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**MATERNAL JUGULAR VEIN**

| Cumulative Bile output   |         |     |          |
| nmol                     | 4.33±0.17 | 3.73±0.15<sup>a</sup> | 4.53±0.18<sup>b</sup> |
| T<sub>1/2</sub>           | 10.7±0.5 | 23.8±1.2<sup>a</sup> | 24.3±1.3 |
| min                      |         |     |          |

The amount of [¹⁴C]-glycocholate ([¹⁴C]-GC) retained by the placenta after administration (250 nmol over 5 min) through the umbilical artery of "in situ" perfused rat placentas in 21-day pregnant rats was measured after 2 or 10 min washing period with [¹⁴C]-GC-free perfusion medium. In two separate sets of animals, values of [¹⁴C]-GC bile output into maternal bile over 3 h after administration of [¹⁴C]-GC through the umbilical artery (250 nmol over 5 min) or through the maternal jugular artery (5 nmol over 5 min) were used to calculate AUC (area under the curve or accumulated output with no extrapolation to infinity) and MRT (mean residence time) by numerical integration using the trapezoidal rule. Half-life time (T<sub>1/2</sub>) of [¹⁴C]-GC in maternal compartment was calculated as Ln2/Ke, where Ke (elimination constant) was 1/MRT (Yamaoka et al., 1978). Groups were Control, and rats undergoing surgical common bile duct obstruction on day 14 and that for the remaining 7 days received the vehicle (OCP group) or 60 µg UDCA/100g b.w./day (OCP+UDCA group). Values are means±S.E.M (n≥5).<sup>a</sup>, p<0.05, on comparing OCP to Controls;<sup>b</sup>, p<0.05 on comparing OCP+UDCA to OCP; <sup>c</sup>, p<0.05 on comparing placental uptake to cumulative bile output. Comparisons were carried out by the Bonferroni method of multiple range testing.
Figure 2
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Figure 4
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**A**

![Graph showing GC uptake vs. GC concentrations (µM)]

**B**

- **Vmax (pmol/30 s/mg protein)**
  - Control
  - OCP
  - OCP + UDCA

- **Km (µmol/L)**
  - Control
  - OCP
  - OCP + UDCA

- **Efficiency of Transport (Vmax/Km)**
  - Control
  - OCP
  - OCP + UDCA

*Significant differences indicated by *.
Figure 5

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(A) Bile Flow (µl/min/g liver)

(B) Bile Acid Output (nmol/min/g liver)

- Control
- OCP
- OCP+UDCA

Time (min)
Figure 6

Serrano et al.

Comparative study of the fetal/maternal serum antipyrine concentration ratio in different maternal and fetal samples: Control, OCP, and OCP+UDCA treatments.

- **Fetal Blood**
- **Jugular Vein**
- **Maternal Liver**
- **Maternal Blood**
- **Placenta**
- **Bile**

*† indicates statistical significance compared to control.

**Legend**:
- ■ Control
- ○ OCP
- ● OCP+UDCA

**Y-axis**: Fetal/Maternal Serum Antipyrine Concentration Ratio

**X-axis**: Time (min)