Excretion of fetal biliverdin by the rat placenta-maternal liver tandem

Oscar Briz, Rocío I. Macías, Maria J. Perez, Maria A. Serrano, and Jose J. G. Marin

1Department of Physiology and Pharmacology, 2Research Unit, University Hospital, 3Department of Biochemistry and Molecular Biology, Laboratory of Experimental Hepatology and Drug Targeting, University of Salamanca, Spain

Submitted 6 July 2005; accepted in final form 27 October 2005

The first products of heme catabolism are Fe, CO, and the blue-green pigment biliverdin (BV) (32). Although the main product is BV-IXα, small amounts of the other three isomers (BV-IXβ, BV-IXγ, and BV-IXδ, depending on the protoporphyrin IX bridge cleaved), in particular BV-IXβ, are also produced in humans and other mammals (40). BV-IXα is further biotransformed to bilirubin (BR) by BV-IXα reductase (BVRα), which is expressed in many tissues (21), including the umbilical artery of in situ perfused rat placentas (5). Using in situ perfused rat placenta, we determined that anti-pyrene, but not BR, is readily able to cross the rat placenta in the mother-to-fetus direction (5). Vectorial properties for transplacental BR transfer are consistent with the moderate increase in serum BR concentrations observed in the fetuses of pregnant rats with hyperbilirubinemia due to common bile duct ligation (17).

How fetal biliary pigments are taken up by the placenta is not well understood. Proteins of the organic anion transporting polypeptide (OATPs) family (10), in particular, human OATP-C/1B1 and OATP8/1B3, have been reported to confer the ability to take up unconjugated BR when expressed in Xenopus laevis oocytes (6). However, the mRNA of OATP-C/1B1 is almost absent in isolated human trophoblast cells, whereas OATP 8/1B3 is clearly expressed in this epithelium (6).

Regarding BV, although this biliary pigment is able to inhibit BR transfer in rat placenta when coadministered through the umbilical artery of in situ perfused rat placenta (5), it has been shown that BV itself is poorly transferred, without prior reduction to BR, across the guinea pig placenta (25).

The aim of the present study was to gain information on the handling of fetal BV by elucidating whether several OATP for its biliary excretion (22). The concentrations of unconjugated BR are higher in fetal than in maternal serum (15, 27). Two facts contribute to this difference: a very active heme catabolism, and hence BR production in the fetus, together with very low BR uridine diphospho-glucuronosyl transferase activity in the fetal liver (14). Thus, during intrauterine life, the placenta is the major route for the excretion of fetal biliary pigments (for a review, see Ref. 19).

The physiological advantages of the transformation of BV into BR have only recently been understood. Thus, generation of endogenous CO, a potent vasodilator, may play a role in the control of local fetal-placental vasculature tone (26). Moreover, in the presence of reactive oxygen species, BR can be oxidized to BV and then converted by BV-IXα back into BR (1). However, beneficial antioxidant properties are limited to low concentrations because BR can also cause irreversible damage or even death when it becomes accumulated and reaches the brain at high levels (9). It has also been suggested that the reduction of BV to BR could have the evolutionary advantage of facilitating the placental excretion of bile pigments by simple diffusion (33). However, in vitro (35) and in vivo (5) studies have suggested that under physiological circumstances the major pathway for BR transfer across the placenta involves carrier-mediated transport across both poles of the plasma membrane of the human trophoblast (35). Moreover, BR does not undergo any major biotransformation during its residence in the rat placenta (5).

Using in situ perfused rat placenta, we determined that anti-pyrene, but not BR, is readily able to cross the rat placenta in the mother-to-fetus direction (5). Vectorial properties for transplacental BR transfer are consistent with the moderate increase in serum BR concentrations observed in the fetuses of pregnant rats with hyperbilirubinemia due to common bile duct ligation (17).

How fetal biliary pigments are taken up by the placenta is not well understood. Proteins of the organic anion transporting polypeptide (OATPs) family (10), in particular, human OATP-C/1B1 and OATP8/1B3, have been reported to confer the ability to take up unconjugated BR when expressed in Xenopus laevis oocytes (6). However, the mRNA of OATP-C/1B1 is almost absent in isolated human trophoblast cells, whereas OATP 8/1B3 is clearly expressed in this epithelium (6).

Regarding BV, although this biliary pigment is able to inhibit BR transfer in rat placenta when coadministered through the umbilical artery of in situ perfused rat placenta (5), it has been shown that BV itself is poorly transferred, without prior reduction to BR, across the guinea pig placenta (25).

The aim of the present study was to gain information on the handling of fetal BV by elucidating whether several OATP...
isoforms involved in the liver uptake of organic anions may play a role in the carrier-mediated uptake of fetal BV by the rat placenta and liver and by measuring the levels of expression of BVRα in the three elements of the fetal liver-placenta-maternal liver trio involved in the handling of fetal BV in native form or biotransformed into BR.

**MATERIAL AND METHODS**

*Chemicals and animals.* Biliverdin (BV) IXα hydrochloride (more than 95% pure, as confirmed by HPLC, see below) was obtained from Frontier Scientific (Logan, UT), which is considered a reliable source of this compound, with sufficient purity for most biomedical investigations (23). 5-carboxy-X-rhodamine triethylammonium salt (ROX) was obtained from Molecular Probes (Leiden, The Netherlands). Estradiol 17β-d-glucuronide (E217βG) and unconjugated bilirubin (BR) was from Sigma-Aldrich (Madrid, Spain). 1H-labeled taurocholic acid (TC, 5 Ci/mmol) was obtained from PerkinElmer Life Sciences (Pacaia & Giralt, Madrid, Spain). Halofuginone (H9251) was obtained from Molecular Probes (Leiden, The Netherlands). 5-carboxy-X-rhodamine triethylammonium salt (ROX) was from Molecular Probes (Leiden, The Netherlands).

Pregnant rats on day 21 of gestation (~370 g) from the University of Salamanca Animal House (Salamanca, Spain) and mature female frogs (*X. laevis*), purchased from Regine Olig (Hamburg, Germany), were used. The animals received humane care as outlined in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Institutional Animal Care and Use Committee Guidebook, 2nd ed., 2002). Experimental protocols were reviewed and approved by the Ethical Committee for Laboratory Animals of the University of Salamanca. Harvesting and preparation of oocytes from *X. laevis* were performed as previously described (6).

*Excretion of biliverdin by the rat placenta-maternal liver tandem.* To carry out these experiments, the rats were anesthetized with sodium pentobarbital (Nembutal N.R.; Abbot, Madrid, Spain) by intraperitoneal injection (50 mg/kg body wt). When needed, polyethylene catheters were inserted into the maternal left jugular vein (for infusion) and the left carotid artery (for sampling). Bile flowing through a polyethylene catheter implanted into the common bile duct was collected in preweighed vials placed on ice and protected from light to prevent the degradation of biliary pigments. Immediately after surgical preparation, the animals were left for an equilibration period of 20 min before starting sample collection.

In situ single-pass perfusion of rat placenta was carried out as previously described in detail (4). Briefly, one of the placentas was exposed and perfused through the umbilical artery using a heparinized saline solution (0.05% heparin, 150 mM NaCl) at 37°C at a constant inflow of 500 μl/min with a peristaltic pump. An incision in the umbilical vein was performed to permit free outflow. Perfusion pressure was measured as an indicator of placental perfusion resistance and was considered to be appropriate when it remained relatively constant and lower than 20 cm H2O throughout the experimental period.

To compare BV handling by the placenta-maternal liver tandem vs. the maternal liver alone or the placenta alone, four separate sets of experiments were carried out, in which BV in 150 mM NaCl at pH 7.8 was administered: 1) 0.1 μmol BV was administered to the mother as a bolus (50 μl/min over 5 min) through the jugular vein. 2) 1.0 μmol BV was single-pass-perfused (500 μl/min over 5 min) through the umbilical artery, followed by continuous perfusion of this placenta with heparinized saline solution throughout the rest of the experimental period. 3) To prevent the biotransformation of the BV by the maternal liver and kidneys once it had been transferred to the mother through the placenta, immediately before administering 1.0 μmol BV through the umbilical artery, the maternal liver and kidneys were rapidly isolated from the maternal circulation using previously replaced ligatures. The viability of these preparations during the experimental period was checked by measuring steady-state concentrations of urea in serum (7) and glucose in blood (Accutrend Sensor kit, Roche Diagnostics, Barcelona, Spain). 4) To investigate the profile of spontaneous accumulation of biliary pigments in serum in this model, similar experiments were carried out, except that vascular isolation of the liver and kidneys was not followed by administration of BV through the umbilical artery. Samples of maternal serum and/or bile were collected periodically to carry out analytical measurements.

To investigate the effect of blocking the maternal biliary excretion of biliary pigments on the expression of BVRα in the fetal liver-placenta-maternal liver trio, complete obstruction of the maternal common bile duct was imposed on day 14 of gestation, and samples of the above tissues were collected on day 21, as described elsewhere (34).

*Measurement of gene expression levels.* To determine mRNA levels by real-time RT-PCR, tissue samples were immediately immersed in the RNA stabilization reagent RNAlater (Qiagen, Izasa, Barcelona, Spain) and stored at −80°C until total RNA was isolated using RNAeasy spin columns from Qiagen (Izasa, Barcelona, Spain). After treatment with RNase-free DNase I (Roche, Barcelona, Spain), RNA was quantified fluorimetrically with the RiboGreen RNA Quantitation kit (Molecular Probes). cDNA was synthesized from 2 μg of total RNA using random hexamers and avian myeloblastosis virus RT (Enhanced Avian RT-PCR kit; Sigma-Genosys, Cambridge, UK), according to the instructions supplied by the vendor.

Real-time quantitative PCR was then performed using AmpliTaq Gold polymerase (Applied Biosystems, Madrid, Spain) in an ABI Prism 5700 Sequence Detection System (Applied Biosystems). The thermal cycling conditions were as follows: a single cycle at 95°C for 10 min followed by 40 cycles at 95°C for 15 s and at 60°C for 60 s. Detection of amplification products was carried out using SYBR Green I. Nonspecific products of PCR, as detected by 2.5% agarose gel electrophoresis or melting temperature curves, were not found in any case. The results of mRNA abundance for each target gene in each sample were normalized on the basis of its 18S rRNA content, which was measured with the TaqMan Ribosomal RNA Control Reagents kit (Applied Biosystems).

The primer oligonucleotide sequences and conditions for measuring the absolute abundances of Oatp1/1a1 (gene symbol Slc21a1/Scl01a1), Oatp2/1a4 (Slc21a2/Scl01a2), and Oatp4/1b2 (Slc21a10/Scl01b2) and rat placental lactogen type II, a specific marker for trophoblastic cells, have been described elsewhere (34). The primer oligonucleotide sequences for BV/Ret were forward primer (position 118–138, GeneBank Accession Number BC078766) 5'-TTG GAG TGG TAG TGG TTG TT-3' and reverse primer (position 288–266) 5'-TCC AAA GAA ATC TGC CGT ACT TC-3'. All primers were designed with the assistance of Primer Express software (Applied Biosystems), and their specificity was checked using BLAST. They were obtained from Sigma-Genosys. Sequencing of the amplified fragments of rat BV/Ret cDNA in maternal liver, fetal liver, and placenta resulted in a similarity of 100% with a previously described sequence (GeneBank Accession Number BC078766).

Western blot analyses on liver and placenta homogenates were carried out in 7.5% SDS-polyacrylamide gels using equal protein loading (150 μg per lane) as confirmed by staining of membranes after transfer with Ponceau Red-S (Sigma-Aldrich). Rabbit polyclonal antibody OSA-400 against BVRα was from Stressgen Bioreagents (bioNova Cientifica, Madrid, Spain). Donkey anti-rabbit IgG horseradish peroxidase-conjugated antibody NA934 and enhanced chemiluminescence reagents were from Amersham Pharmacia Biotech (Freiburg, Germany).

*Uptake studies using X. laevis oocytes.* Using the PolyATtract mRNA Isolation System purchased from Promega (Innogenetics, Barcelona, Spain), poly(A) mRNA to be injected into the oocytes was isolated from total RNA, obtained from rat liver or placenta as described above. The synthesis of cRNA of rat Oatpl/1a1, Oatp2/1a4, and Oatp4/1b2 for injection into oocytes was performed using recombinant plasmids containing the open reading frame DNA of these transporters, all kindly supplied by Dr. Peter J. Meier, Dr. Bruno Stieger, and Dr. Bruno Hagenbuch (Zurich University Hospital, Switzerland). These plasmids were isolated from Escherichia coli using
the Qiagen Plasmid Mini Kit (Izasa) and further linearized with restriction enzymes. Capped and poly(A) tailed cRNAs were synthesized using the T7 mMessage mMachine Ultra kit (Ambion; bioNova Cientifica).

X. laevis oocytes were microinjected with TE buffer (1 mM EDTA, 10 mM Tris, pH 8.0) either alone, or for determining nonspecific uptake and binding of the substrates, or containing the following amounts of RNA: 25 ng mRNA from rat liver or placenta or 7 ng cRNA of each OATP. Oocytes were used 2 days after RNA injection, when, on the basis of preliminary experiments on the time course of the functional expression for these carriers, the uptake rate was highest (data not shown).

Uptake studies were carried out using groups of five oocytes per data point. The data represent the results of at least three separate sets of RNA injections. Oocytes were washed with substrate-free uptake medium (100 mM choline chloride, 2 mM KCl, 1 mM CaCl2, 1 mM MgCl2, and 10 mM HEPES/Tris, pH 7.0) and incubated with 100 μM [3H]TC or 100 μM BV at 25°C for 1 h. Uptake was stopped by the addition of 4 ml ice-cold uptake medium. The oocytes were washed three more times before being collected and were placed in vials individually (to measure radioactive substrate by liquid scintillation) or in groups of two oocytes (to increase the absorbance signal in HPLC analysis of BV uptake).

Analytical and statistical methods. Bile flow was determined gravimetrically. BR concentrations in bile were determined by a modification of the diazotized sulfanilic acid coupling method (18) and BV by HPLC as described below. To determine BV and BR in serum, both pigments were extracted (2), and subsequently dissolved in methanol. The mixture was treated to transform BR glucuronides into the corresponding methyl esters, as described elsewhere (5), evaporated under nitrogen and dissolved in 200 μl lysis/extraction solution (methanol containing 50 pmol ROX as internal standard for HPLC) to be analyzed by HPLC using 1 ml/min flow rate in a solvent gradient system. The initial mobile-phase composition was 100% phosphate buffer (2.5 mM, pH 7.2). After 1 min, the solvent was progressively changed to 50% methanol and 50% phosphate buffer over 2 min. This elution system was maintained for 1 min before starting a 5-min linear change to 100% methanol, which was maintained until the end of the runtime (20 min). Measurement of absorbances at 378, 450, or 567 nm wavelength for BV, BR, or ROX, respectively, were used for peak detection.

To measure BV in oocytes, these were placed in vials (in groups of two oocytes) and treated with 150 μl lysis/extraction solution and sonicated for 5 min in an ice-chilled bath. Then, oocyte lysates were centrifuged at 20,000 g for 5 min, and the supernatants were used to measure BV by HPLC.

Results are expressed as means ± SD. Regression lines were calculated by the least-squares method. To calculate the statistical significance of the differences between groups, the paired Student’s t-test or the Bonferroni method for multiple-range testing were used, as appropriate.

RESULTS

To evaluate the overall handling of BV by the placenta—maternal liver tandem, 1.0 μmol BV was administered through the umbilical artery of in situ perfused rat placenta in a single-pass perfusion system (Fig. 1). Only 1.6% of the pigment was recovered in maternal bile—almost all of it (99%) biotransformed into BR—over the following 90 min [15.5 (SD 3.2) nmol BR plus 0.13 (SD 0.05) nmol BV]. Moreover, in a different set of experiments, 1.0 μmol BV was administered through the umbilical artery of in situ perfused rat placenta, just after vascular isolation of the maternal liver and kidneys (Fig. 2). The result was that a peak in serum BV concentrations appeared before the general deterioration, which occurred after 20 min, as monitored by increasing levels of urea and decreasing glucose concentrations in maternal serum. A transient peak of BV concentrations was followed by a delayed (5–10 min), and progressive, increase in unconjugated BR, but not in conjugated BR (data not shown), concentrations in maternal
serum (Fig. 2). The magnitude of the increase in serum concentrations for BV and BR is consistent with the amount of BR secreted into bile when BV was administered to animals with intact liver and kidney circulation. No detectable spontaneous accumulation of any of these pigments was observed in animals up to 20 min after vascular isolation of the liver and kidneys when BV was not administered through the umbilical artery (BV was not detected and BR concentrations remained below 0.3 μM) (Fig. 2).

To measure the proportion of BV converted into BR by the maternal liver after crossing the placenta, a dose of 10% of that administered through the umbilical artery (i.e., 0.1 μmol) was administered directly into the maternal circulation by injection through the jugular vein (Fig. 3). Approximately 80% of this amount was eliminated in bile over the following 90 min as a mixture of BR and BV. Most of the secreted pigments (80%, 61.7 (SD 3.6) nmol) were found to be BV biotransformed into BR, but a smaller, although considerable, amount (20%, 15.7 (SD 4.0 nmol) was secreted in bile as BV.

To study the ability of several OATP isoforms to transport BV, these transporters were expressed in X. laevis oocytes, and BV uptake was measured in a HPLC system. ROX was used as an internal standard, because BV and ROX showed similar (98%) recoveries from oocytes undergoing the lysis and extraction protocol (data not shown), similar retention time in HPLC, and low interferences between them in the recording of absorbance chromatograms (Fig. 4A). Using this method, the

Fig. 3. Time course of the increase in BV (●) and BR (○) bile output after the administration of 0.1 μmol BV as a 5-min bolus through the jugular vein of 21-day pregnant rats. Values are means ± SD from five experiments. The inset depicts the schematic representation of the experimental model.

Fig. 5. Sodium-independent uptake of [3H]taurocholic acid ([3H]TC) and biliverdin-IXα (BV-IXα) by X. laevis oocytes injected with 7 ng cRNA of Oatp1/1a1, Oatp2/1a4, or Oatp4/1b2. Oocytes were incubated in uptake medium (100 mM choline chloride, 2 mM KCl, 1 mM CaCl2, 1 mM MgCl2, and 10 mM HEPES/Tris, pH 7.0) containing 50 μM [3H]TC or 100 μM BV-IXα at 25°C for 60 min. BV uptake was measured by HPLC, following an incubation-lysis-extraction protocol carried out with two oocytes in each measurement. Values are expressed as means ± SD from at least 15 determinations and represent net uptake, calculated by subtracting the uptake by oocytes injected with TE buffer without cRNA. *P < 0.05, on comparison using the Bonferroni method for multiple-range testing with the uptake of the same substrate by oocytes injected with Oatp1/1a1 cRNA.
presence of endogenous BV in X. laevis oocytes was detected. This is not surprising because this pigment has been found previously in eggs from very different species (22). The technique permitted linear detection of BV over a large concentration range (Fig. 4B) and a clear discrimination between non-specific uptake in noninjected oocytes (Fig. 4C) and transport-mediated uptake. Indeed, injection of oocytes with mRNA from rat placenta resulted in an enhanced uptake of BV (Fig. 4D). When carrier-mediated uptake (uptake in injected oocytes minus that in noninjected oocytes) was calculated, the ability of three members of the OATP family, involved in the uptake of organic anions by the rat liver to induce sodium-independent uptake of TC (Oatp4/1b2 > Oatp1/1a1 > Oatp2/1a4) was observed (Fig. 5). This was more marked for BV (Oatp1/1a1 > Oatp2/1a4 > Oatp4/1b2) (Fig. 5). A similar ability to enhance overall uptake of TC was induced by injection of mRNA from either rat placenta or maternal liver, and both were similarly inhibited by E217βG (Fig. 6), a substrate shared by several OATP isoforms (10). Moreover, mRNA from placenta induced a significantly higher BV uptake than that from maternal liver (Fig. 6). E217βG also induced a dramatic inhibition of BV uptake in both cases (Fig. 6). It should be noted that in experiments carried out with oocytes injected with total mRNA, overall uptake was the result of an uptake-efflux balance, as export pumps were probably also expressed.

To investigate the relevance of OATPs as transporters responsible for BV uptake by the rat placenta, their expression levels in this organ were determined by measurement of their absolute abundance of mRNA (Fig. 7A). In all cases, the expression levels were markedly lower in placenta than in maternal liver. Moreover, the most abundant isoform in the placenta was Oatp1/1a1. When these values were corrected by the amount of trophectoderm, using the rat placental lactogen type II mRNA as a marker, the abundance of mRNA for Oatp1/1a1 was 7- to 8-fold higher than that for Oatp2/1a4 and Oatp4/1b2 (Fig. 7B).

To study the potential contribution of fetal and maternal livers and the placenta to the biotransformation of fetal BV into BR, the relative abundance of BVRα in these organs was determined by real-time quantitative RT-PCR, after confirming that no nontarget products were generated during amplification, as revealed by the appearance of a single peak in the DNA melting curves and 2.5% agarose gel electrophoresis of amplified products (Fig. 8A).

To compare the relative expression of this enzyme in different tissues, the abundance of its mRNA and protein was considered per gram of tissue (Fig. 8B) and by Western blot analysis per fixed amount of total protein (Fig. 8B, inset), respectively. The highest relative expression was found in fetal liver, followed by the placenta and the maternal liver.
evaluate the total relevance of each organ in the overall activity of this enzyme in the whole animal, the relative abundance of mRNA was corrected by the weight of each organ (Fig. 8C). The highest total amount of mRNA was found in maternal liver, followed by the fetal liver and the placenta.

Cholestasis imposed in pregnant rats during the last week of pregnancy induced a marked increase in maternal serum total BR concentrations from 0.14 (SD 0.03) mg/dl in controls (n = 6) to 3.02 (SD 0.51) mg/dl in the group with cholestasis (n = 6; P < 0.05), together with an upregulation of BVRα in maternal liver (Fig. 8, B and C). However, the repercussion on fetal serum BR concentrations was less marked [from 0.31 (SD 0.08) mg/dl in the group with cholestasis (n = 12; P < 0.05)] and was not accompanied by any effect on the expression of BVRα in fetal liver (Fig. 8, B and C). The expression of BVRα in the placenta, which is exposed to blood from both compartments, was not affected by maternal cholestasis either (Fig. 8, B and C).

**DISCUSSION**

Many studies have been conducted recently to shed light on the role of plasma membrane transporters in the rat and human placental handling of endogenous substances and xenobiotics (16, 20, 29). The present study provides direct evidence that several of the OATPs expressed in rat placenta and maternal liver are able to take up BV from the fetal and maternal circulations. Among them, Oatp1/1a1 and Oatp4/1b2 are abundantly expressed in the maternal liver, and, together with the contribution to a lesser extent of Oatp2/1a4, may play an important role in BV uptake from the maternal blood. As regards the placenta, Oatp1/1a1, owing to its ability to transport BV and its high expression compared with that of Oatp2/1a4 and Oatp4/1b2, is probably the most important of these three transporters contributing to BV uptake by this organ from fetal blood. Nevertheless, other transporters may also participate in this function.

The observation that when mRNA from liver or placenta was injected in X. laevis oocytes, the uptake by these cells was higher for BV than for TC, is likely due to the fact that Na⁺-independent uptake, which was the type measured in the present study, could be more important for BV than for TC. It should be noted that at the basolateral surface of rat hepatocytes, TC uptake is mainly governed by the very efficient Na⁺-taurocholate cotransport polypeptide (Ntcp, gene symbol Slc10a1) (11). Moreover, the expression of export pumps may differently reduce the net amount of TC and BV found in the cells after 60 min incubation.

In vivo experiments, only a minor part of the amount of BV that was taken up by the rat placenta was rapidly released toward the mother, followed by a delayed release of BR. This suggests that the transport of BV from the trophoblast toward the mother is very poor and/or that placental biotransformation of BV into BR is very efficient. Indeed, our results confirm the

---

Fig. 8. A: validation of conditions to measure biliverdin IXα reductase (BVRα) mRNA levels by SYBR Green I-based real-time quantitative RT-PCR. Representative plot of the first negative derivative curves calculated from plots of the SYBR Green I-DNA fluorescence of the amplified PCR products vs. changes in temperature (dF/dT). The resulting peak corresponds to the melting temperature of an amplified specific BVRα fragment. The inset shows the amplified PCR product visualized on 2.5% agarose gel after electrophoresis. Lane 1: standard DNA ladder; PCR was carried out without DNA template (lane 2) or with cDNA obtained by RT of rat kidney RNA (calibrator) as template (lane 3). B: relative abundance of BVRα mRNA in different organs determined by SYBR Green I-based real-time quantitative RT-PCR. Values represent means ± SD from the percentage of expression per gram of tissue. Abundance of BVRα mRNA was compared with the calibrator used in all reactions. Expression levels were normalized on the basis of the content of 18S rRNA measured in the same sample. The inset shows representative Western blot analysis of liver and placenta homogenates from the same experimental groups. C: values of BVRα mRNA abundance as calculated per whole organ. In six pregnant rats (cholestasis group), the common bile duct was ligated on day 14 of pregnancy. Six (maternal liver) or twelve (placenta and fetal liver) different preparations for each tissue in each group were obtained on day 21 of pregnancy, and RT-PCR was performed in triplicate for each sample. *P < 0.05 on comparison of control with maternal cholestasis; †P < 0.05 on comparing maternal liver and placenta with maternal liver; ‡P < 0.05 on comparing placenta with fetal liver. Comparisons were carried out by the Bonferroni method for multiple-range testing.
expression in rat placenta of BVRα, whose enzymatic activity has previously been reported (25). Moreover, expression levels of this enzyme in the placenta, which were higher than that in maternal liver, are consistent with the observed extensive biotransformation of BV into BR during placental transit. The fact that the expression of BVRα in fetal liver was even higher than in the placenta, suggests that an important part of endogenous BV produced by the fetus could be transformed into BR by the fetal liver before being taken up by the placenta.

Although several human OATPs, namely OATP-C/1B1 and OATP8/1B3, are able to transport BR (6, 8) and could play a role in BR uptake by the placenta (6), little is known about the fate of this pigment, or that of BV, during the rest of transplacental transit (19).

Inside the trophoblast, BR is probably partly bound to lipids and proteins such as glutathione S-transferase (39). Functional studies have suggested that from there, BR would be exported across the apical pole of the trophoblast via an ATP-dependent mechanism (35). Whether one or several isoforms of multidrug resistance-associated proteins (MRPs), such as MRP1 and MRP2, which are expressed in human (38) and rat placenta (34, 37), are involved in this process is not known. Canalicular MRP2 plays a key role in the secretion into bile of mono- and di-glucuronides of BR (12). However, owing to the low UDP-glucuronosyl transferase activity of the fetal liver and the absence of placent al biotransformation of unconjugated BR during transplacental transfer, when this compound is administered through the umbilical artery of in situ perfused rat placentas (5), MRP2 is not expected to play an important role in BR transfer across the placenta. Moreover, the excretion of dicarboxylate organic anions related to BV into rat bile is also mediated by MRP2-independent mechanisms (24), suggesting that this export pump is not responsible for the poor release of BV from the trophoblast into the maternal blood across the apical pole of this epithelium.

Like MRP2, MRP1 is also able to perform ATP-dependent transport of bilirubin glucuronides (12). Although indirect evidence has suggested that MRP1 may also be involved in the efflux of unconjugated BR from human choriocarcinoma BeWo cells (28), its role in BR and BV transport across the placenta remains to be elucidated.

An interesting observation of the present study was that the impairment in maternal bile secretion induced an upregulation of BVRα in the maternal liver. This probably enhances the ability of this organ to generate BR from BV as part of the redox cycle, BR being an important defense mechanism against oxidative stress (1), such as that caused by maternal cholestasis (30). However, probably because of the lower exposure of fetal liver to compounds accumulated in maternal blood (17), the expression of BVRα in fetal liver was not affected by maternal cholestasis (30). Nevertheless, the expression of several OATPs and MRPs can be induced in rat placenta by maternal cholestasis (34), which in addition to these events also causes enhanced oxidative stress and apoptosis (31). This results in an overall impairment of the ability of this organ to transfer cholephilic organic anions, such as bile acids, from the fetus to the mother (34).

In conclusion, our results suggest that among transporters involved in fetal BV uptake by the rat placenta, several OATPs, in particular Oatp1/1a1, may be involved. Once in the placenta, and before transfer to the mother, BV is extensively converted into BR by BVRα, which is highly expressed in this organ. The small amount of BV that reaches the maternal blood is efficiently taken up, probably in part by Oatp1/1a1, Oatp2/1a4, and Oatp4/1b2, and biotransformed into BR, which joins fetal BR transferred as such by the placenta, to be eliminated mainly through secretion into bile by the maternal liver.

ACKNOWLEDGMENTS

The authors thank Dr. P. J. Meier, B. Stieger, and B. Hagenbuch (University Hospital, Zurich, Switzerland) for his generous supply of recombinant plasmas containing the cDNA of rat OATPs. Thanks are also due to L. Muñoz, J. F. Martin, J. Villoria, N. Gonzalez, and E. Vallejo for care of the animals. Secretarial help by M. Hernandez, technical help by E. Flores and the revision of the English spelling, grammar, and style of the manuscript by N. Skinner are also gratefully acknowledged by the authors.

GRANTS

This study was supported in part by the Junta de Castilla y León (Grant SA013/04) Spain, as well as the Ministerio de Ciencia y Tecnología, Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (Grant BF12003–03208) and Fondo de Investigaciones Sanitarias (FIS/CP03/00093). The group is a member of the Network for Cooperative Research on Hepatitis, Instituto de Salud Carlos III, FIS (Grant G03/015).

REFERENCES

Handling of Biliverdin by the Rat Placenta


