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Concise Review

Molecular bases of the excretion of fetal bile acids and pigments through the fetal liver-placenta-maternal liver pathway

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Abstract

Since the excretion of potentially toxic cholephilic organic anions (COAs) produced by the fetus, such as bile acids and biliary pigments, cannot be performed by the fetal liver alone, the placenta and the maternal liver must play a key role collaborating in this function. COAs are transported across the plasma membranes of fetal and maternal hepatocytes and trophoblastic cells via similar carrier proteins. OATPs (organic anion-transporting polypeptides), mainly OATP1B1 and OATP1B3 are involved in COA uptake across the basal membrane of adult hepatocytes and trophoblastic cells. Certain OATPs may also play a role in COA efflux from fetal hepatocytes toward the fetal blood and from the trophoblast to the maternal blood. Either unmodified or biotransformed during their transit across the placenta, COAs are transferred to the maternal blood by MRPs (multidrug resistance-associated proteins), such as MRP1, MRP2 and MRP3. BCRP (breast cancer resistance protein) may also be involved in this step. Under physiological circumstances, fetal COAs are taken up by the maternal liver, which eliminates them across the canalicular membrane via MRP2 and BSEP (bile salt export pump). However, when normal biliary excretion is not possible, the accumulation of COAs, in particular in the fetal liver, placenta and maternal liver trio, induces oxidative stress and apoptosis, which has noxious repercussions on normal fetal development and even challenges pregnancy outcome. Treatment of pregnant rats with ursodeoxycholic acid, even though maternal hypercholanemia is not corrected, prevents oxidative damage and the subsequent deleterious effects on the placenta and fetal liver.

Key words: Bilirubin, cholephilic Organic Anions, Cholestasis, Oxidative Stress, Pregnancy, Ursodeoxycholic Acid.

A large variety of structurally unrelated compounds can be secreted into bile by the adult liver. For some of them, the hepatobiliary pathway is by far the major one for their elimination from the body. These are commonly termed as cholephilic compounds, which include several endogenous anions, such as bile acids (BAs) and biliary pigments, mainly biliverdin and bilirubin. The present review focus on the molecular bases of the mechanisms responsible for the elimination of these cholephilic organic anions (COAs) during the fetal life through the excretory pathway constituted by the fetal liver, placenta, and maternal liver trio (Figure 1).

Some COAs are taken up by the liver and excreted into bile without undergoing major biotransformation. Detoxification is therefore carried out only by transport mechanisms: i.e., phase 0 (uptake) and phase III (secretion). In contrast, other COAs undergo chemical modifications during their transcellular residence due to detoxification mechanisms involving oxidation/reduction reactions (phase I) and/or conjugation with polyatomic groups (phase II).[1]

Although some COAs, such as unconjugated bilirubin, are able to enter cells by simple diffusion,[2] most COAs are taken up mainly via plasma membrane carrier proteins (for a review, see [3]). Thus, in adult hepatocytes, this process is performed in part by members of the fam-

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Abbreviations:
BAs, bile acids; BP, biliary pigments; COAs, cholephilic organic anions; ICP, intrahepatic cholestasis of pregnancy; OCP, obstructive cholestasis during pregnancy; UCB, unconjugated bilirubin.

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ily of organic anion-transporting polypeptides (OATPs), including OATP-A or OATP1A2, according to the new nomenclature recently adopted by the HUGO Gene Nomenclature Committee[4] (gene symbol *SLCO1A2*), OATP-C or OATP1B1 (*SLCO1B1*) and OATP-8 or OATP1B3 (*SLCO1B3*). Although all three isoforms have been shown to be able to transport BAs (for a review, see [5]) and, some of them, also unconjugated bilirubin (UCB).[6,7] owing to the low expression of OATP1A2 in normal adult liver cells its role in COAs uptake is probably lower than that of OATP1B1 and OATP1B3.

Another transport system involved in COAs uptake by the liver that is not present in lower vertebrates, and hence is probably phylogenetically more modern than OATPs, is the Na^+-taurocholate-cotransporting polypeptide (NTCP, gene symbol *SLC10A1*).[8,9] This carrier belongs to a family of sodium-dependent co-transporters that also includes the intestinal bile acid transporter (IBAT, gene symbol *SLC10A2*), which plays a major role in active bile acid uptake by the intestine and is also expressed in cholangiocytes and renal proximal tubular cells.[10] A negligible expression of NTCP has been found in rat[11] and human[12] placenta, which is consistent with functional evidence that suggest that the carrier-mediated uptake of BAs[13] across the basal plasma membrane of the trophoblast - as happen for UCB[14] - is not sensitive to sodium gradients.

Other transporters of the SLC22A family are able to transport organic anions (OATs) or organic cations (OCTs), and they hence participate in the uptake by the liver of a large variety of compounds,[15] including some COAs and their derivatives.[16] In this respect, it should be noted that OCT3 (gene symbol *SLC22A3*) is particularly abundant in human placenta.[17]

In adult hepatocytes, phase III processes are performed by efflux pumps, most of them belonging to the superfamily of ATP-binding cassette (ABC) proteins. These ATPases use the energy of ATP hydrolysis to actively transport a large number of different substrates out of cells. The following ABC proteins are located in the canalicular plasma membrane: the P-glycoprotein, also termed multidrug resistance protein (MDR1; gene symbol *ABCB1*), which is probably involved in the secretion of organic and inorganic cations;[18] the sister of the P-glycoprotein or bile salt export pump (BSEP; gene symbol *ABCB11*), which is the major mechanism of bile acid secretion into bile;[19] the isoform 2 of the multidrug resistance-associated protein (MRP2; gene symbol *ABCC2*), which exports conjugated bilirubin, dianionic sulfated or glucuronated metabolites, including BAs, and drugs, such as cisplatin,[20,21] and the breast cancer resistance protein (BCRP; gene symbol *ABCG2*), which can transport steroids, including BAs, with higher efficiency for sulfated derivatives.[22,23]

The level of expression of MRP1 (*ABCC1*) and MRP3 (*ABCC3*) in the basolateral plasma membrane of adult hepatocytes is very low, but this can be markedly increased in response to cholestasis[24-26] and endotoxemia.[27] Up-regulation of these pumps in pathological conditions might favor the elimination from liver cells of potentially toxic COAs, which would subsequently be excreted by the kidney when they cannot be secreted into bile.[28]

Handling of cholephilic organic anions by the fetal liver

In healthy adult humans, the major BAs are primary BAs - those synthesized directly by the liver from cholesterol -, and secondary BAs - those resulting of modification of primary BAs by intestinal bacteria -.[29] Surprisingly, although fetal intestinal function is very poor, and hence BAs seem unnecessary, at least for digestive purposes, as from very early on in gestation the fetal liver is able to carry out bile acid synthesis,[30] and indeed serum BA concentrations are higher in fetuses than in their mothers in both rats[31] and humans.[32-34]

However, the fetal hepatobiliary excretory pathway is not yet fully functional.[30] Therefore, the fetal liver must transfer these COAs to the placenta, which would eliminate them toward the mother. A minor contribution by the fetal kidney, which can secrete them into the amniotic fluid, also exists.[35]

Although not only of hepatic origin, bilirubin is also produced by the fetus, and contributes to enhanced concentrations of this COA in fetal serum.[34,36] It is not known how the COAs produced by the fetal liver exit from hepatocytes. However, at least some OATPs are believed to behave as bi-directional transporters[37] and their expression has been detected in fetal liver. Using real-time quantitative RT-PCR the mRNA levels of several transporters have been measured recently at different timepoints during rat gestation.[11] Except for Oatp4a1, the abundance of mRNA in fetal liver is lower than in adult liver. Thus, although an efficient bile acid transporter, Oatp1b2 (previously named Oatp4), is up-regulated during the last third of gestation its mRNA levels increase from only 1% to 10% of values found in adult liver. Among the substrates transported by Oatp4a1[38] and its human ortholog, OATP4A1 (previously named OATP-E),[39] are some BAs.

Regarding the possibility that active export of COAs across the basolateral plasma membrane of fetal hepatocytes might be mediated by MRPs, it is noteworthy that mRNA levels for Mrp1 in fetal liver are three-fold higher than in adult liver.[11]

Placental transfer of bile acids

Based on the lipid nature of BAs and the existence of concentration gradients across the placenta, it has long been accepted that simple diffusion is the major route for their transplacental transfer. However, at physiological pH both in blood and within the cells most BA molecules

are in anionic form, which are poorly diffusible across cell membranes.[40] Moreover, simple diffusion would permit BA transfer in both directions, which would reduce efficiency of the overall process and would not prevent the risk of the increased fluxes of these compounds from the maternal blood that may occur under pathological circumstances accompanied by hypercholanemia.

In fact, BA transfer in the mother-to-fetus direction also exists, because secondary BAs are present in the fetal BA pool, even though the bacteria accounting for their production are absent in the fetus. The transplacental gradient for secondary BAs is inverse to that of primary species: i.e., secondary BAs are more abundant in maternal than in fetal serum.[34] However, this transfer is very low, as demonstrated by the fact that maternal cholestasis, induced in rats by complete obstruction of common bile duct, which is accompanied by marked hypercholanemia, results in only a moderate increase in BA concentrations in fetal serum.[41] The existence in the trophoblast, as the major mechanism of BA transfer across the placenta, of transport proteins, some of which have unidirectional transport characteristics, accounts for the vectorial properties of the overall process (for a review, see figure 1).[42]

The first evidence for the existence of carrier proteins involved in BA transport across the human placenta came from functional experiments using plasma membrane preparations obtained from the basal, or fetal-facing,[13,43] and apical, or maternal-facing,[44-46] poles of the human trophoblast.

The placental phase 0 for fetal COAs involves the uptake of these substances across the basal plasma membrane. For fetal BAs, this step is carried out by sodium-independent anion exchange.[13] Since there is a bicarbonate-gradient from maternal-to-fetal blood and since bicarbonate has been shown to activate BA transport across this membrane, it is likely that BA:bicarbonate exchange would mediate this process,[47] which is not similarly effective, and hence partly selective for the different molecular species of BAs.[48] This is probably involved in establishing the differences in the composition of fetal BA pool as compared to that of the mother.[34] This transport system is able to transport COAs other than BAs,[49] suggesting that it could play a role in the detoxification of other compounds from the fetal compartment.

Functional characteristics suggest that members of the OATP family could be involved in this transport process. The mRNA for some of these proteins in rat[11,50] and human[7] placenta has been detected. In human placenta, the expression levels of OATP1B3 were found to be higher than those of OATP1B1 and OATP1A1 - all of them able to transport BAs make it smaller.[7] Although OATP4A1 (previously OATP-E) is believed to be a thyroid hormone transporter, it also transports certain BAs and is highly expressed in human placenta.[39] The ortholog Oatp4a1 (previously Oatp12) is also abundantly expressed in rat placenta.[11] However, the overall role of OATP4A1 in fetal BA uptake is not clear, since this carrier has been predominantly detected at the apical surface of the human syncytiotrophoblast.[39] The expression levels of the BA carriers Oatp1a1, Oatp1a4 and Oatp1b2 in rat placenta are very low under physiological conditions,[11] but they are up-regulated during maternal cholestasis and, even more so when pregnant rats are treated with ursodeoxycholic acid.[50] Moreover a role in this function of other transporters cannot be ruled out. Thus, interesting candidates are OATP2B1 (previously OATP-B), which has been localized at the basal membrane of human trophoblast,[51] and Oatp2b1 (previously Oatp9), whose mRNA is also detected (although at less than 10% of that found in adult rat liver) in rat placenta.[11] However, there is some controversy regarding substrate-specificity for both orthologs. While rat Oatp2b1 seems to be able to transport taurocholate,[52] no ability to transport BAs is observed when OATP2B1 (previously OATP-B) is expressed in *Xenopus laevis* oocytes.[51]

As shown in functional studies,[46,53] phase III or the export of BAs from the trophoblast toward the maternal blood, is probably mainly carried out by ATP-dependent transport systems. However, a role for ATP-independent mechanisms has been also suggested.[44,45] The latter could involve OATPs, such as OATP4A1.

Regarding ATP-dependent pumping mechanisms, several ABC proteins expressed in placenta may be involved (for a review, see [54]). Among these transporters, several multidrug-resistance associated proteins (MRPs) with a known ability to transport BAs whose expression in placenta has been detected include MRP1, MRP2 and MRP3 in human placenta[55] and Mrp1, Mrp2 and Mrp3 in rat placenta.[50] At least in rats, these three transporters are markedly up-regulated during maternal cholestasis,[50] which could enhance the defensive barrier against the inverted gradient of BAs which may favor the entry of these compounds into the trophoblast and which these transporters may return to the maternal blood. Indeed, whereas rat common bile duct ligation resulted in an increase in serum BA concentrations of 220 μM in the mother, these were only approximately 30 μM in their fetuses.[50] Another candidate to be involved in this function is MRP4, which is expressed in basolateral membrane of human hepatocytes, and is able to mediate efflux of glutathione by cotransport with anionic BAs.[56] Moreover, at least in rats, Mrp4 mRNA levels have been found to be about 20-fold higher in placenta than in normal liver, although lower - approximately 30% - than those in kidney.[57]

Although BSEP mRNA has been detected in human[12,58] and rat placenta,[11] its abundance is so low that a major physiological role for this protein in BA transport across the placenta is unlikely.

In spite of the high expression of BCRP in placenta,[59] which accounts for one of the names of this protein, i.e., ABC placental protein (ABCP), and its recently described

ability to transport BAs,[23] its relevance in overall BA transport across this organ has not been evaluated yet.

Placental transfer of biliary pigments

Fetal hemeoxygenase catalyzes the convertion of protoporphyrin IX into biliverdin, mainly biliverdin IXα plus carbon monoxide (CO).[60,61] Hemeoxygenase (HO) consists of two isoenzymes: HO-1 (inducible and mainly expressed in liver and spleen) and HO-2 (constitutive and widely distributed throughout the body). In placenta, HO-2 is expressed in syncytiotropoblast and endothelial cells, where CO release may play a role in the control of placental perfusion.[62] The activity of biliverdin reductase-alpha, which is detectable in several organs[63] including the placenta,[64] accounts for the biotransformation of biliverdin – a non-toxic and water-soluble green pigment – into bilirubin – a potentially toxic and poorly water-soluble yellow pigment –, mainly bilirubin IXα. In the adult, UCB is efficiently taken up by the liver via saturable and concentrative mechanisms that are mediated by transport proteins.[65] In hepatocytes, UCB is mono- or di-conjugated with glucuronic acid by the bilirubin uridine diphosphate-glucuronosyl transferase-1A1[66] to generate water-soluble derivatives that are secreted into bile by MRP2.[20] When the excretion of bilirubin into bile is impaired, up-regulation of MRP3 permits the regurgitation of bilirubin glucuronides into the plasma across the basolateral plasma membrane of hepatocytes.[67]

Serum concentrations of UCB are higher in fetal than in maternal blood.[34,36] This can be explained in terms of the following two additive features: on one hand, the fetus has a high rate of bilirubin production due to active haemoglobin F turnover during late gestation[68] and an elevated fragility of blood red cells.[69] On the other hand, the activity of UCB-conjugating enzyme in fetal liver is very poor, as is the hepatobiliary excretory pathway.[70] In fact, in fetal gallbladder bile only appreciable amounts of bilirubin IXβ have been found, probably due to the fact that this is much more water-soluble than the IXα isomer, and hence conjugation is not mandatory for its secretion into bile.[71] Therefore, for fetal bilirubin IXα it is transplacental elimination that constitutes the major excretory pathway.

Owing to the existence of a fetal-to-maternal concentration gradient and in view of the physical-chemical lipophilic characteristics of UCB, it has also long been accepted that one of the advantages of the evolutionary selection of the transformation of biliverdin into bilirubin was the need for a route for heme catabolites to cross the placenta, which is easily carried out by UCB via simple diffusion, but not by biliverdin.[64] Moreover, when bilirubin was administered *in utero* to fetal guinea pigs[72] and monkeys[73-75] the transfer of UCB from the fetal to the maternal circulation was rapid, while that of the conjugated derivative was almost absent, indicating that only UCB is able to cross the placenta.

Nevertheless, although a diffusional pathway of UCB across plasma membranes does exist[2,14] and may contribute to transplacental UCB transfer, functional *in vitro* evidence suggest that the major pathway for UCB transport across the basal and apical membranes of the human trophoblast is carrier-mediated[14] (Figure 1). Studies carried out using *in situ* perfused rat placenta have found that UCB is transported from the fetal to the maternal compartment via a process that can be inhibited by several COAs, which is not consistent with simple diffusional transfer.[76] Moreover, UCB was not biotransformed during its transit across the rat trophoblast.

When antipyrine, a highly diffusible compound,[77] was intravenously administered to pregnant rats, serum antipyrine concentrations readily became similar in mothers and fetuses.[76] However, transfer from the maternal serum to the fetal compartment of UCB, when co-administered with antipyrine, was very poor.[76] The following additional observation suggests that UCB transfer has vectorial properties and hence further supports the concept that this process is not mainly due to simple diffusion: as happens with BAs, when complete obstructive cholestasis was induced in pregnant rats, this resulted in a marked increase in bilirubin concentrations in maternal serum, whereas those in fetal serum were only moderately affected.[50]

Moreover, the normal placental capacity for the transport of bilirubin in the fetus-to-mother direction is limited. Indeed this is exceeded in situations of enhanced destruction of fetal blood red cells, where an elevation in fetal serum bilirubin concentrations often precedes the development of antenatal anemia, which is associated with fetal hemolytic disease.[78]

Functional and molecular biology studies have suggested that the transport systems responsible for the uptake of UCB from fetal blood are sodium gradient-independent and ATP-independent mechanisms,[14] probably including members of the OATP family, and in particular OATP1B3.[7] In contrast, efflux from the trophoblast toward the maternal blood involves transporters activated by ATP hydrolysis. Owing to the presence of MRPs in the human[55] and rat[50] trophoblast, it is tempting to suggest a role for these proteins in UCB transport. Thus, indirect evidence obtained in experiments on BeWo human choriocarcinoma cells led the authors to suggest a role for MRP1 in this process.[79] However, the actual role of MRPs in UCB transport across the placenta mechanism remains obscure.

Repercussions of maternal cholestasis on the placenta and fetal liver

The excretory pathway for COAs described above is of great relevance because when it is impaired the repercussions on the normal development of the fetus or even on the fate of gestation may be dramatic. Intrahepatic cholestasis of pregnancy (ICP) is a reversible form of

cholestasis that may develop during late pregnancy and usually resolves soon after delivery. For the mothers, this condition is usually benign since it is only associated with certain discomfort due to pruritus. However, ICP is frequently the cause of premature delivery and increased risk of fetal mortality during the third trimester of pregnancy in patients suffering this disease (for a review, see [80]). Moreover, the severity of fetal complications is proportional to the magnitude of maternal hypercholanemia.[81] An inverse relationship between maternal serum concentrations of BAs and the functional activity of the ATP-dependent bile transporter located at the apical membrane of human trophoblast has also been reported.[82]

To experimentally induce accumulation of COAs in maternal blood of laboratory animals, complete obstructive cholestasis during the last third of pregnancy and the lactation period (OCP) has been imposed on pregnant rats in a series of studies. Elevated serum BA concentrations were detected both *in utero*[41] and at birth[31] in offspring born from OCP rats. Congenital alterations in hepatobiliary function were detected in young animals, characterized by a partial impairment in the ability of the liver to secrete COAs, whereas the BA-induced biliary secretion of phospholipids, but not that of cholesterol, was markedly enhanced.[31,83] These alterations have been associated in part with delayed maturation of the mechanisms involved in hepatocyte transcytosis,[84] as well as with the presence of multilamellar bodies in the bile canaliculi, which might act as plugs to hinder bile flow.[83] In contrast, no alterations in the expression of basolateral transporters involved in COAs uptake[85,86] or in the efficiency of ATP-dependent BA transport across the canalicular membrane[87] were found in these animals. However, Mrp1 was markedly up-regulated and cholesterol transporters ABCG5/ABCG8 were down-regulated.[86]

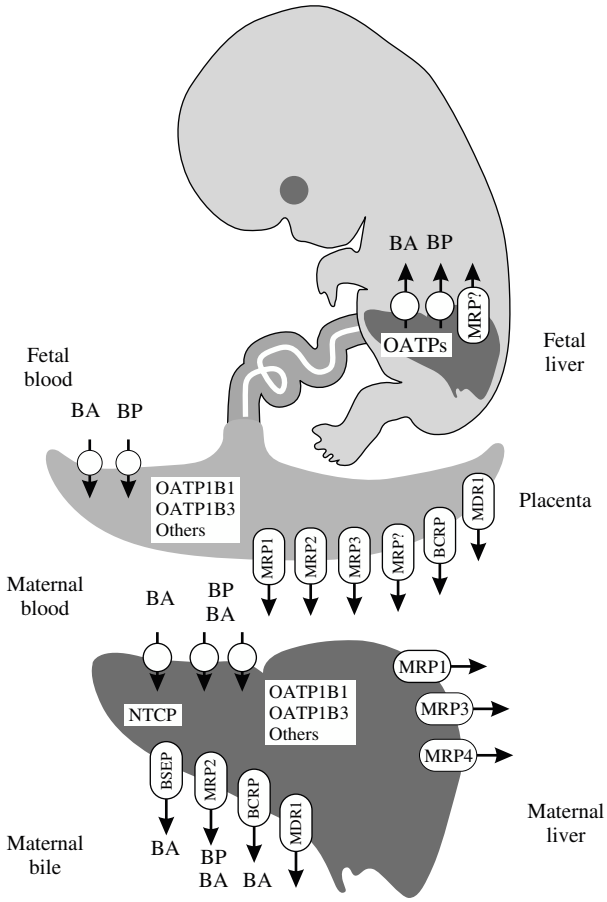

Figure 1. Schematic representation of the plasma membrane carriers involved in the normal uptake/secretion of bile acids (BA) and biliary pigments (BP) by adult and fetal hepatocytes and trophoblastic cells. NTCP, Na^+-taurocholate-cotransporting polypeptide; OATPs, organic anion-transporting polypeptides; MDR1, multidrug resistance protein; BSEP, bile salt export pump; MRPs, multidrug-resistance associated proteins; BCRP, breast cancer resistance protein.

An interesting issue that has recently been addressed is how the accumulation of COAs can affect fetal and placental tissues. Since the placenta is exposed to high concentrations of BAs at the maternal side, it is reasonable to assume that the well-known cytotoxicity associated with the most hydrophobic BAs may become an insult for the trophoblast.[88] Indeed, OCP induces impairment of the placental antioxidant system and oxidative damage. These alterations are accompanied by enhanced activation of the mitochondrial pathway of apoptosis. Treatment of pregnant rats with ursodeoxycholic acid has a beneficial effect on the placenta by partly preventing these changes.[89]

Nevertheless, the placental barrier for COAs is not completely abolished since, as has been commented above, despite the existence of marked maternal hypercholanemia, OCP causes only a moderate accumulation of COAs in the fetal compartment. However, this is sufficient to induce marked oxidative damage and apoptosis in the fetal liver.[90] Treatment of pregnant rats with ursodeoxycholic acid has beneficial effects by lowering the exposure of the fetus to toxic BAs, restoring the levels of glutathione in fetal liver, preventing lipid peroxidation and protein carbonylation, and correcting pro-apoptotic alterations in the Bax-α/Bcl-2 ratio.[90]

OCP-induced alterations in fetuses and placentas may be responsible for both reduction in the number of fetuses per pregnancy and post-natal impairment in hepatobiliary function. Both changes can be prevented by treatment of pregnant rats with cholestasis with ursodeoxycholic acid.[86]

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