Intrahepatic cholestasis of pregnancy: Changes in maternal-fetal bile acid balance and improvement by ursodeoxycholic acid
Abstract

Intrahepatic cholestasis of pregnancy (ICP) is a disease characterized by generalized pruritus and biochemical cholestasis that appears typically during the last trimester of gestation. The most predictive and accurate markers for diagnosis and follow-up of ICP are increased total bile acid levels (above 11.0 µmol/L), enhanced cholic acid percentage (above 42%) and decreased glycine/taurine bile acid ratio (below 1.0). Although essentially benign for the mother, evidence associates ICP with fetal poor prognosis resulting from increased transfer of bile acids from mother to fetus, who showed reduced ability to eliminate bile acids across the placenta. Those conditions lead to an accumulation of bile acids in the cord blood serum, meconium and amniotic fluid that may account for a diminished fetal well-being and sudden intra-uterine death by ICP. Ursodeoxycholic acid (UDCA) treatment was shown to reduce the bile acid content in the fetal compartment, while restoring the ability of the placenta to carry out vectorial transfer of these compounds towards the mother, decreasing bile acid levels in maternal serum and its passage to the fetus. In addition, UDCA administered to the mother also lowers the amount of bile acids present in colostrum without either increasing the UDCA concentration or causing major changes in lithocholic acid levels, further supporting the safety of UDCA in late pregnancy. Therefore, it is tempting to indicate UDCA as a first choice therapy for ICP as much as relevant aspects of fetal outcome may also be improved. This review focuses on the altered bile acid profiles in maternal and fetal compartments during ICP and its recovery by UDCA administration. Further elucidation of the precise mechanisms of action of UDCA and its therapeutic potential in improving fetal prognosis could result in the approval of UDCA for ICP treatment.

Key words: Cholestasis, Pregnancy, Ursodeoxycholic acid, Bile acid.

Introduction

Cholestasis, whether resulting from hereditary or acquired liver diseases, is one of the most common manifestations of impaired bile flow. Intrahepatic cholestasis of pregnancy (ICP) is a condition of unknown etiology characterized by generalized pruritus and biochemical cholestasis, which occurs predominantly during the last trimester of pregnancy. However, appearance of ICP as early as the 10th week of pregnancy has been reported. Pruritus generally starts in palms and soles. A mild jaundice may be noticed in 20% of patients after the onset of itching while a slight elevation in the concentration of conjugated bilirubin (17.1 ± 1.7 µmol/L) is observed in 90% of ICP women. Among serum liver enzymes, alanine aminotransferase is the most sensitive test, with 2 to 10 times increased values and its efficiency was referred to be over 90%, while γ-glutamyltranspeptidase is the least elevated. A more specific biochemical parameter of ICP is the rise of serum bile acids with levels comprised between 12.4 and 219.4 µmol/L as compared to the upper normal limit in late gestation of 11 µmol/L. Shortly after delivery, symptoms disappear and biochemical parameters of liver cell function return to normal.

The incidence of ICP is high in Chile (14%) and Bolivia (9.2%), but less common in Europe where prevalence rates of 1 to 1.5% have been described for Portugal, Sweden, Poland and Finland. Prevalence is higher in twin than in single pregnancies and several clinical and experimental observations evidenced a primary role of estrogens and progesterones in ICP. Genetic predisposition also plays a key role in the pathogenesis of ICP and incidence increases within the same family. Current research indicates that mutations of the MDR3 gene encoding the canalicular phosphatidylcholine translocase may in some cases predispose to ICP, justifying the raised γ-glutamyltranspeptidase in 20 to 40% of the patients. However, other characteristics of ICP...
suggest that exogenous factors may superimpose on the hormonal and genetic factors. This is indicated by seasonal changes in the appearance of the disease, with a higher number of cases in January, pointing to a greater incidence during the winter.5,21 Recent studies also link ICP to low serum selenium levels.22,23 In conclusion, ICP seems to result from combined and multivariate effects.

Although essentially benign to the mother, quality of life can be impaired due to itching, and urinary tract infection, postpartum hemorrhage, and fat and vitamin K malabsorption are common complications during ICP.11,24,25 The most serious consequences of ICP are increased fetal distress, premature deliveries and perinatal mortality and morbidity24,26,27 (Table I). Meconium staining of amniotic fluid is considered a sign of poor prognosis for the fetus.27,28 Interruption of pregnancy is considered mandatory and urgent, mainly in an already mature fetus, when fetal distress is detected and there is risk of sudden death in utero.4 Therefore, ICP should be considered a high-risk condition, and an early and accurate identification of high-risk pregnancies together with an appropriate medical intervention might improve fetal outcome.

Several treatments, such as cholestyramine, phenobarbital, dexamethasone, S-adenosyl-L-methionine, and epomediol failed to improve pruritus, biochemical abnormalities, or fetal prognosis during ICP.29,30 The most efficacious current medical management that improves maternal condition and might prevent the perinatal complications of ICP is ursodeoxycholic acid (UDCA) administration.36-42 Thus, as soon as ICP is diagnosed, UDCA administration coupled with close maternal-fetal surveillance is indicated.

**Bile acids in fetal pathophysiology**

Bile acids easily go through the placenta to fetal compartments and also to the amniotic fluid. Usually, there are higher concentrations of bile acids in fetal than in maternal serum and the main transfer of bile acids across the placenta occurs towards the mother43,44 (Figure 2). B. During ICP, in addition to the increased levels of bile acids, the efficiency of the ATP-dependent transport mechanisms is enhanced (Figure 2). Provided they are not unidirectional, both changes may facilitate the passage of bile acids from the mother to the fetus, and hence counterbalance or even overcome bile acid flux across the placenta in the physiological direction, which is mediated in part by ATP-independent systems and whose efficiency is reduced during ICP.45 Furthermore, the same authors have shown that vectorial bile acid transfer from fetus-to-mother is also impaired, thus contributing to an accumulation of bile acids in the fetal compartment. This accumulation may be associated with the occurrence of increased fetal distress by ICP. Actually, Laatikainen and Tulenheimo46 found a correlation between total serum bile acids and the incidence of meconium and fetal distress. In contrast, others were unable to find a direct correlation between bile acids in any compartment and fetal distress.47 Nevertheless, total bile acids in maternal serum greater than 50 µmol/L, or superior to 25 µmol/L in fetal cord serum, are associated with diminished well-being47 and levels particularly elevated are referred to cause sudden fetal death.7

Autopsy specimens in cases of intrauterine fetal loss from ICP are consistent with death from acute intrauterine anoxia.27 Meconium and bile acids, especially cholic acid, have been indicated to induce vasoconstriction of human placental choriocytic veins in vitro,8,49 as well as causing acute umbilical vein constriction.49,50 Thus, there is some experimental evidence that bile acids are implicated in the mechanisms triggering fetal asphyxia in pregnancies complicated by ICP. In addition, it was recently shown that taurocholate (0.3 and 3 mM), the main bile acid during ICP, causes a decrease in the rate of contraction of rat cardiomyocytes and loss of synchronous beating.51 This data corroborates a direct role of bile acids in the sudden intrauterine death by ICP.

Therefore, the decrease in bile acid levels induced by UDCA therapy in ICP patients, besides reversing maternal symptoms, may also improve fetal outcome. Palma et al.52 confirmed that patients who received UDCA had had their deliveries closer to term and less frequent fetal distress as compared to non-treated patients.

**Relevance of UDCA as a therapeutic option for ICP**

During the three last decades, several clinical studies have established UDCA as a promising therapeutic option in a variety of cholestatic liver diseases, including prima-
UDCA normalizes bile acid profile in maternal serum

The most specific and sensitive biochemical test of ICP is serum bile acid levels, which may reach values 100 times above normal. Concentrations of total bile acids in normal pregnant women are consistently lower than 11.0 µmol/L, while values from 12.3 to 219.4 µmol/L or even 290 mmol/L are encountered in women with ICP. Evaluation of serum total bile acids is recommended, not only to diagnose but also to monitor ICP. The upper reference limits for both cholic and chenodeoxycholic acids in healthy pregnant women vary from 1.5 µmol/L to 4.2 µmol/L. In contrast, cholic acid values may increase up to 170 µmol/L during ICP, while chenodeoxycholic acid represent 3 to 4 times less. Taurocholic acid is the predominant species presenting values between 2.4 and 119.8 µmol/L, accounting for 38.1 ± 1.9% of the total bile acids as compared to 23.6 ± 1.4% for glycocholic acid (concentrations from 2.0 to 55.8 µmol/L). This shift towards a more extensive conjugation with taurine (glycine/taurine bile acid ratio of 0.8 ± 0.1, as compared to 1.4 ± 0.1 in normal pregnancies) is pointed as a marker of ICP. In healthy women, either pregnant or not, cholic acid is never higher than 45% of serum total bile acids, but accounts for 60 to 70% during cholestasis. Therefore cholic/chenodeoxycholic acid ratio is always greater than one. As previously noticed the beneficial effect of UDCA therapy may be associated with its ability to promote changes in the hydrophobic-hydrophilic balance of the bile acid pool, by increasing hydrophilicity. In a study with 15 ICP patients treated with UDCA (14 mg/kg/day), the concentration of total bile acids decreased significantly (P<0.01) from 68.4 ± 16.1 µmol/L at baseline to 20.8 ± 5.1 µmol/L during therapy, in agreement with reports by others. Individually, the most significant alteration is the reduction of hepatotoxic bile acids, replacement of hepatotoxic bile acids, immune modulation, cytoprotective mechanisms by preventing apoptosis, cholestatic hepatocytes by inducing hepatobiliary excretion and insertion of the transport protein MRP2 in the canalicular membrane (Figure 1). In addition to the beneficial effect on the functionality of the maternal liver, UDCA therapy restores the ability of the placenta to carry out vectorial bile acid transfer, contributing to prevent an excessive accumulation of bile acids in the fetal compartment during ICP.

This highly hydrophilic bile acid reduced pruritus, amino-transferases, and serum levels of total bile acids when administered to patients with ICP. Patients usually receive UDCA at oral applications of 450 mg/day, 1.66 12-16 mg/kg/day or 1.5 to 2 g/day (20-25 mg/kg/day) in three or four daily divided doses. The drug is well tolerated and seems to be completely safe for the mothers or their babies. Pregnancy outcome and perinatal prognosis were improved in ICP patients treated with UDCA compared to placebo.

Table I. Perinatal outcome in both control and cholestatic pregnancies.

<table>
<thead>
<tr>
<th></th>
<th>Control (25)</th>
<th>Control* (28)</th>
<th>ICP (13)</th>
<th>ICP (25)</th>
<th>ICP (27)</th>
<th>ICP (28)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n=42)</td>
<td>(n=79)</td>
<td>(n=117)</td>
<td>(n=44)</td>
<td>(n=83)</td>
<td>(n=79)</td>
</tr>
<tr>
<td>Spontaneous labor</td>
<td>3(7.9%)</td>
<td>37(46.8%)</td>
<td>17(38.6%)</td>
<td>27(33%)</td>
<td>60(75.9)</td>
<td></td>
</tr>
<tr>
<td>Fetal distress</td>
<td>1 (1.3%)</td>
<td>22 (18.8%)</td>
<td>12 (14%)</td>
<td>6 (7.6%)</td>
<td>11 (14%)</td>
<td></td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>6 (7.6%)</td>
<td>4 (3.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>7(15.8%)</td>
<td>3 (3.8%)</td>
<td>2 (1.7%)</td>
<td>8 (18.4%)</td>
<td>6 (7.6%)</td>
<td></td>
</tr>
<tr>
<td>Meconium passage</td>
<td>6 (7.6%)</td>
<td>19 (16.2%)</td>
<td></td>
<td>37 (45%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Neonatal death</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Low birth weight (&lt;2500 g)</td>
<td>3(7.9%)</td>
<td>14(31.6%)</td>
<td></td>
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</tbody>
</table>

*Women with a history of unexplained fetal death; ICP, intrahepatic cholestasis of pregnancy. References are indicated in parentheses.
may be clinically relevant since it has been reported to cause fetal distress, as previously mentioned.

This change in the composition of the bile acid pool is accompanied by a significant increase in the concentration and proportion of UDCA from 0.6 ± 0.2 µmol/L and 1.4 ± 0.6% to 5.9 ± 1.9 µmol/L and 24.7 ± 2.3%, respectively, at baseline and during treatment. These results resembled those of 0.1-4.8 µmol/L obtained in another set of patients under similar dosage (12-16 mg/kg/day). However, serum conjugated UDCA levels may reach concentrations as high as 16.5 ± 1.8 µmol/L when dosages of 1.5 to 2 g/day are used. Experimental studies have not shown any teratogenic or adverse effects of UDCA on pre- or post-natal development in rats.

Only a small proportion of total bile acids is detected in the unconjugated fraction, even during UDCA administration, despite its increase from 7.2 ± 1.3% at baseline to 12.8 ± 1.6% during treatment. Since UDCA may be converted to lithocholic acid, known to induce growth retardation and malformations in rat embryos, some concern has been expressed regarding its safety in pregnancy and the treatment is contraindicated in France. Nevertheless, data has shown that the serum lithocholic acid concentration is maintained during UDCA administration (1.7 ± 0.5 µmol/L at baseline, and 1.2 ± 0.2 µmol/L during therapy), despite an increase in its proportion (7.4 ± 1.3% vs 3.3 ± 0.5%, P<0.01). Therefore, although under continued evaluation, UDCA might be the first-line therapy for ICP.

**Passage of bile acids into colostrum decreases following UDCA administration**

Infant exposure to high levels of bile acids may be particularly risky, since enterohepatic circulation is immature at birth, ileal and hepatocyte transport mechanisms are impaired, and serum bile acid concentrations are increased, conditions that may aggravate neonatal complications in ICP. Excretion of bile acids in colostrum is enhanced following ICP. In fact, when bile acid excretion in colostrum collected from 16 lacting ICP women, within 72 h postpartum was compared to five lacting healthy women, elevation of total bile acids levels (23.3 ± 14.8 µmol/L vs 0.7 ± 0.2 µmol/L, P<0.01) and of cholic acid concentrations (19.0 ± 13.1 µmol/L vs 0.6 ± 0.2 µmol/L, P<0.01) were found. The low levels of cholic acid in colostrum from control lacting women are similar to those reported in other studies, even for breast milk. Cholic acid predominates either in colostrum from normal or ICP lacting women. It is worthwhile to mention, however, that values of total bile acids as higher as 100 µmol/L may arise in colostrum of ICP patients and be absorbed by breast-feeding infants. Therapy diminishes the excretion of total bile acids in colostrum to 5.7 ± 2.5 µmol/L and cholic acid is the most reduced one (3.6 ± 1.5 µmol/L). Accumulations of UDCA (0.3 ± 0.2 µmol/L) and lithocholic acid (0.01 ± 0.01 µmol/L) in colostrum are irrelevant and toxicity is not expected to occur in nursing infants.

**Altered patterns of bile acids in meconium are unchanged by UDCA therapy**

Data on meconium bile acid composition in neonates from ICP patients is scant. In a recent study it was reported a considerable meconium elevation of total bile acids (13.5 ± 5.1 µmol/g vs 2.0 ± 0.5 µmol/g) and cholic acid (8.4 ± 4.1 µmol/g vs 0.8 ± 0.3 µmol/g) in newborns from women with ICP, indicating placental transfer of these compounds from mother-to-fetus. This is reinforced by the presence of deoxycholic and lithocholic acids in meconium, which are supposed to be of maternal origin since they are products of bacterial metabolism and fetal colon is sterile. Thus, these secondary bile acids must reach the fetus by transfer across the placenta. Continued ingestion by the fetus of amniotic fluid may also contribute to the increase of bile acids in meconium, due to its high content in cholic acid.
In addition to cholic, chenodeoxycholic, deoxycholic and lithocholic acids, significant amounts of unusual bile acids were equally identified,83,87,88 resembling composition of fetal gallbladder bile.89 Interestingly, during ICP there is a decrease in the rate of UDCA excretion (in normal conditions) and the diminished intestinal absorption, greatly reducing the beneficial effects of the therapy at this level. Nevertheless, it must be emphasized that in order to restrain the placental passage of bile acids and the accumulation of these compounds in meconium, it is important to decrease bile acid levels in maternal serum and thus UDCA treatment must be initiated as soon as the diagnosis of ICP is made.

**UDCA reduces the levels of bile acids in amniotic fluid**

Bile acids in amniotic fluid may originate from the mother, the fetus, or both.86 Levels of primary bile acids are dramatically elevated in the amniotic fluid during ICP, indicating the maternal compartment as the first source. In addition, the presence of unusual bile acids, from which the more abundant are polyhydroxylated bile acids, suggest that fetal liver under conditions of higher bile acid level attempts to excrete bile acids into urine by increasing their polarity.91

Whether bile acids reach the amniotic fluid by diffusion through umbilical cord or placental membranes or by fetal urine and meconium remains to be established. Interestingly, both cholic and chenodeoxycholic acids are more elevated in the amniotic fluid from normal pregnancies than in the maternal serum.37

During ICP, cholic acid elevation is the main contributor to the increased content of amniotic fluid in primary bile acids (Table II) with mean values from 12.5 ± 4.7


Bile acid levels diminish in umbilical cord blood when UDCA is given to ICP women

Concentrations of total bile acids in umbilical cord serum of premature and term neonates range from slightly to 2-3 times higher than in maternal blood and increase even more in fetus of an early gestational age. Primary bile acids predominate and the cholic/chenodeoxycholic acid ratio is close to 1.0. The elevation of both cholic and chenodeoxycholic acids in the fetal blood as compared to maternal serum suggests immaturity of liver function during the fetal period, as seen during the first months after birth. On the other hand, the placenta also seems to maintain this concentration difference.

Levels of primary bile acids increase up to 2 and 5 times in the cord blood serum during ICP, although elevation in maternal serum is even more notorious. Mean ratio between levels of cholic and chenodeoxycholic acids augment to 2.0 reflecting, although to a less extent, the typical elevation of this ratio in the ICP women. Serum total bile acids are greatly increased in babies from ICP complicated gestations compared with values from those of healthy mothers. Cholic acid levels of 14.0 ± 2.9 µmol/L obtained in the same study are close to the value of 21.9 ± 5.9 µmol/L reported for another group of 9 babies (Table II). The higher cholic acid proportion (51.8 ± 7.8%), in contrast with that of chenodeoxycholic acid (25.2 ± 8.3%), appears to result from its greater distribution in maternal serum, which is considered a characteristic feature in ICP patients. The diminished glycine/taurine ratio found in the fetal bile acid pool (0.8 ± 0.2) also seems to be determined by an equivalent ratio in maternal serum.

UDCA therapy induces an increase in the fetal serum proportion of this bile acid (from 1.9 ± 2.0% to 12.5 ± 8.4%, P<0.05), accompanied by a significant decrease in total bile acid concentration (14.8 ± 2.7 µmol/L, P<0.05), and in cholic acid proportion (39.4 ± 13.3%, P<0.05), and by a trend to normalization in the glycine/taurine ratio (1.2 ± 0.4, P<0.10). UDCA concentration increases from 0.57 µmol/L in control, and from 0.40 µmol/L in non-treated patients, to 1.83 µmol/L in ICP women receiving UDCA. In contrast, a more recent report indicates a lower accumulation of conjugated UDCA (0.9 ± 0.14 µmol/L), despite the high-dosage of 1.5 to 2.0 g/day used. The authors justify the differences based on an increase in the mother’s bile acid secretion by such high-dose UDCA treatment strategy. Both studies show that important improvements are achieved for cholic and chenodeoxycholic acids (Table II). Therefore, improved fetal prognosis during maternal treatment with UDCA, in the course of ICP, is probably related with the UDCA capability to normalize the bile acid profile in the umbilical cord serum.

**Conclusion**

During ICP high bile acid levels reach the fetal compartment having maternal serum as the first source. Increased bile acid concentrations in serum and colostrum of ICP patients determine an enhanced flux of bile acids from mother to fetus and its absorption by breast-feeding
infants, respectively, with cholic acid representing the major species. Whether bile acids reach the fetus through umbilical cord or placental membranes, remains to be established. To the accumulation of bile acids in fetal serum, meconium and amniotic fluid contribute the enhanced transplacental systems of bile acid transport towards the fetus on one hand, and the reduced ability of the fetus to eliminate bile acids on the other hand. UDCA therapy restores the maternal-fetal bile acid balance during ICP, is not harmful to the fetus and above all may hamper stillbirths and preterm labors. It is hoped that research in this field yields insights that will result in the approval of UDCA as the first line treatment option for ICP patients.

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