CHAPTER 7

General Discussion and Future Perspectives

1.Introduction

Unconjugated bilirubin is a neurotoxic compound and severe unconjugated hyperbilirubinemia is associated with the development of bilirubin-induced neurotoxicity; kernicterus spectrum disorders (KSD). KSD can result in permanent neurological damage including cerebral palsy, sensorineural deafness, gaze abnormalities, and even death. Unconjugated hyperbilirubinemia is a common condition in infants. It primarily occurs during the first two weeks of life and especially premature infants are at risk for bilirubin-induced neurotoxicity.

The aim of the research performed in this thesis was to optimize and explore new possibilities for the management of neonatal hyperbilirubinemia, using a translational approach. Figure 6 schematically depicts the different aspects of bilirubin physiology and treatment of hyperbilirubinemia that have been studied in the different chapters of the thesis. In short, in chapter 2, we reviewed conjugated bilirubin (CB) transporters ATP-binding cassette (ABC) transporters ABCC2 and ABCC3 and discussed their role in bilirubin metabolism, cholestasis and drug disposition. Also, we addressed their (post)transcriptional regulation by nuclear receptors.

In chapter 3 we focussed on the diagnosis and monitoring of neonatal hyperbilirubinemia by describing the postnatal course of free unbound bilirubin (UCBfree) and the ratio between UCBfree and total serum bilirubin (TSB), the (UCBfree/TSB) ratio. Both UCBfree and the UCBfree/TSB ratio increase after birth, with a peak on postnatal day 4. Also, male infants show higher UCBfree levels on this day. Whereas gestational age and birth weight are significant predictors of the TSB and UCBfree/TSB ratio, these clinical variables do not affect UCBfree levels. Although these parameters may more accurately

reflect the risk of bilirubin neurotoxicity, UCBfree measurements are not (yet) available in daily clinical care.

Fluorescent tube (FT) phototherapy has been described to induce oxidative stress and oxidative DNA damage. In chapter 4 and 5 we evaluated whether Light Emitting Diode (LED) phototherapy has similar side effects. We showed that irradiances up to 100μW/cm^2/nm did not induce the oxidative DNA damage markers 8-hydroxy-2'deoxyguanosine (8-OHdG) or gamma-H2AX in Gunn rats and that irradiances up to 35 μW/cm^2/nm did not induce 8-OHdG in preterm neonates. These results mitigate concerns about potential detrimental side effects regarding oxidative stress of high irradiance LED phototherapy.

In chapter 6 we investigated the potential of therapeutic bile acids ursodeoxycholic acid (UDCA) and obeticholic acid (OCA) to prevent or treat neonatal hyperbilirubinemia in two animal models of this disease. Both compounds proved to be highly effective in decreasing plasma and brain unconjugated bilirubin (UCB). These effects are partially mediated by induction of intestinal UDP-glucuronosyltransferase (UGT)1A1 expression. Also, our data indicate that activation of farnesoid-X-receptor (FXR), the bile acid sensing nuclear receptor, might play a role in these effects.

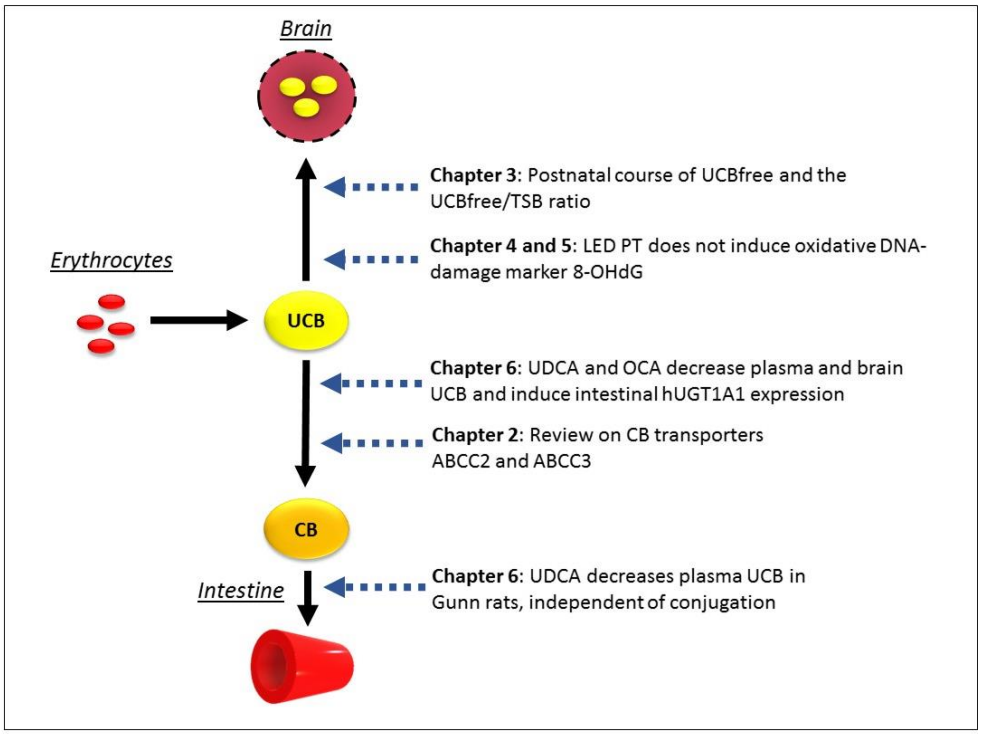

Figure 6: Overview of neonatal bilirubin metabolism and the targets of the various studies in this thesis

2. Free bilirubin

Current guidelines on the treatment of hyperbilirubinemia use TSB-based treatment thresholds for decisions on starting or (de-)intensifying therapeutic interventions. However, TSB is a poor predictor of bilirubin-induced neurological dysfunction (BIND) and, especially in sick low birth weight (LBW) infants [1,2], kernicterus can occur at relatively low TSB levels [3]. This is not surprising, since only the non-albumin bound UCB fraction, UCBfree, is able to enter and deposit in the brain. Although TSB and UCBfree correlate with each other, phototherapy does not decrease UCBfree to the same extent as TSB. This is especially true for infants <28 weeks, in which phototherapy decreases TSB, but not UCBfree [4]. Also, Heghy et al.[5] reported that the correlation between

UCBfree and TSB is completely lost when UCBfree exceeds the threshold of 21 nM. These levels are not uncommon in premature infants and frequently found in our cohort [6,7].

TSB levels have remained the gold standard for decades, since routine UCBfree measurements are not clinically available. Only one technique, the Arrows method, which was developed in Japan, has been approved by the FDA, but the technique has not become commercially available outside Japan [8,9]. Also, the Arrows method has several technical limitations, including the need for a 42-fold sample dilution, whereas sample dilution has been shown to intrinsically alter bilirubin-albumin binding[10]. This method uses only one peroxidase concentration, whereas results are influenced by peroxidase concentrations [11]. The peroxidase method that we used, as described by Jacobsen et al. [12] measures both TSB and UCBfree, and has been the most commonly used method. However, the technique is relatively complex, time-consuming and requires relatively large plasma volumes, and is therefore not routinely used. In 2012, Huber et al.[13] introduced an UCBfree probe, that measured UCBfree using a fluorescently labelled fatty acid binding protein mutant and was able to determine UCBfree within minutes in 8 µL plasma. In adult human plasma, supplemented with different bilirubin concentrations and bilirubin-albumin complexes, concentrations equalled the peroxidase method of Jacobsen. In 2018, this UCBfree probe was used in preterm neonates, following a similar set-up as we did in the study described in Chapter 3 [5]. Although both TSB and UCBfree levels were lower when compared to our data, the course of UCBfree was comparable, with maximal values on day 4. A disadvantage of this novel probe, however, has been the need for a 25-fold sample dilution. However, a newer version is under development that is to be able to measure UCBfree in 5 µl whole blood [5]. The development of such an easy, affordable and accessible UCBfree measurement will greatly enhance the possibilities for further research and might pave the way for routine clinical measurements of UCBfree.

It allows for studying risk factors that increase UCBfree and identify drugs or other endo- and exogenous substances that interfere with the bilirubin-albumin binding, on a large scale.

We characterized the postnatal course of UCBfree in preterm neonates. We conclude that UCBfree levels vary considerably, despite a low incidence of classic hyperbilirubinemia risk factors and despite a low incidence of comorbidity and hypoalbuminemia [7]. Despite successful TSB-based phototherapy management, UCBfree levels in our cohort frequently exceed neurotoxic levels, as defined in literature [2,5,14]. However, we are currently awaiting neurodevelopmental outcome data of infants in our study, to assess the specific neurotoxic thresholds using our method of UCBfree determination. Nevertheless, the large variation of UCBfree with frequently exceeding neurotoxic levels, underlines the need for better understanding of the clinical variables that affect UCBfree and bilirubin-albumin binding. Ultimately, this will need to result in UCBfree-based treatment guidelines that can base treatment thresholds on bilirubin-induced neurotoxicity risk rather than TSB levels.

Apart from TSB, the total amount of UCBfree theoretically depends on the available intravascular albumin pool, and the bilirubin-to albumin binding affinity. We show that in our cohort, UCBfree is not predicted by the albumin concentration and that the bilirubin/albumin ratio has no additional value over TSB in predicting UCBfree. These results are in line with previous work showing that bilirubin/albumin-based guidelines do not improve neurodevelopmental outcomes [15,16]. Alternatively, the UCBfree/TSB ratio which combines the concentration of the potentially neurotoxic compound UCBfree, with the total vascular bilirubin concentration, was reported to be the best predictor of abnormal automated auditory brainstem responses (AABR). Its correlation to abnormal AABR is even stronger than that of UCBfree alone, whereas TSB is not correlated at all [1]. Thereby, the UCBfree/TSB ratio is suggested to be the best

BIND predictor. Although the smallest infants are subjected to the strictest TSB-thresholds, we show that the UCBfree/TSB ratio is higher in smaller infants. This indicates that phototherapy does not decrease UCBfree to the same extent as TSB in these infants, and confirm the data of Hegyi et al. (2017) [4], which shows that phototherapy decreases both TSB and UCBfree in infants >28 weeks, but does not decrease UCBfree in infants <28 week gestational age. These findings support the concept that TSB treatment thresholds may not be as appropriate as assumed, especially not in infants <28 weeks.

Chapter 3 shows that male infants are predisposed to higher UCBfree peak values on day 4. These data are in line with earlier findings of Amin et al.[14] who show a higher risk for bilirubin-induced auditory toxicity in hyperbilirubinemic male compared to female infants and also with the findings of Seidman et al. [17] who showed, only in males, a significant correlation between severe neonatal jaundice and lower IQ at 17 years. In the 2004 AAP-guidelines, male gender is included as a minor risk factor, but no stricter thresholds have so far been implemented for male infants [18]. We propose that the new UCBfree probe should be used to further delineate the male predisposition to high UCBfree levels and BIND, alongside with already described interactions. Especially the relationship between UCBfree and increased free fatty acids plasma levels, caused by intravenous lipid treatment, should be critically evaluated in clinical trials[19,20].

3. LED phototherapy

Phototherapy plays a pivotal role in current neonatal care and is a very effective and convenient therapy of neonatal hyperbilirubinemia. Even today, more than 60 years after its introduction, it is still being improved. Recent studies have focussed on different wave lengths of phototherapy, on intermittent phototherapy, and on high(er) irradiance phototherapy [21]. The latter has become widely available with the introduction of LED phototherapy. LED-based

devices are gradually replacing fluorescent tube devices, since they use less energy and are able to emit high-irradiance light without significant heat production. Current treatment guidelines however, still adhere to multi-day low or moderate irradiance treatment over shorter high irradiance treatment, whereas Vandborg et al.[22] showed that even at irradiances of 55 μW/cm^2/nm, no saturation point for its efficacy was reached. Also, shorter bouts of intermittent phototherapy at high irradiances have proven as effective as continuous treatment and require less interference with the mother-child interaction and result in less disturbance of the circadian rhythm[23].

Although high-irradiance LED phototherapy >50 μW/cm^2/nm has been available for some years, it is not broadly implemented in clinical care. Although the reason for the reluctant implementation is not investigated, in recent years questions have arisen regarding the safety of phototherapy. Short term side effects primarily include an increase in insensible water loss and potential disturbances in electrolyte homeostasis. However, these side effects are mainly caused by the heat produced by FT phototherapy or halogen photothearpy and seem to hardly play a role in LED phototherapy[24]. Also, two studies suggest increased mortality in preterm infants following phototherapy [25-28]. One study was conducted in the 1970s and mentions no details on the phototherapy protocol or devices. The Network trial (2008)[25,26], reports an increased mortality among infants <750g birth weight under 'aggressive' phototherapy. However, both mortality findings need to be put into perspective, since although mortality seemed increased, the differences were not found to be significant. Furthermore, whereas 'aggressive' might insinuate a higher irradiance, it referred to the TSB treatment threshold at which phototherapy was initiated or stopped (5 vs. 8 mg/dL). Due to the stricter threshold, phototherap was started at an earlier postnatal age and phototherapy duration was longer. With regards to phototherapy-type or intensity, the study protocol did not dictate anything and various phototherapy lamps could have been used,

including heat-emitting FTs [29]. Irradiances ranged from 15-40 μW/cm²/nm, but the effect of irradiance was not studied. Nevertheless, aggressive phototherapy caused a significant improvement of neurodevelopmental outcome.

Regarding long-term side effects, LED phototherapy is hardly studied, but FT phototherapy has been epidemiologically associated with diabetes type I, asthma and childhood epilepsy [30-33]. Furthermore, several studies have reported induced levels of oxidative stress and DNA-damage after phototherapy (Table I). All studies investigating the effect FT phototherapy on oxidative stress and DNA damage in neonates describe enhanced levels, but only three studies reported on LED phototherapy, with conflicting results [34-36]. Therefore, results are difficult to interpret; the compared doses of FT and LED phototherapy were not the same or irradiances were not accurately monitored. Therefore, the effect of LED phototherapy on oxidative stress and DNA damage has remained largely unclear.

The studies described in chapters 4 and 5 are the first to report on the effects of intensive LED phototherapy on oxidative stress and DNA damage with irradiances up to 100 μW/cm²/nm. To assess potential genotoxic effects, we used 8-OHdG and γH2AX as markers for DNA oxidation and DNA damage, respectively. The first is virtually completely excreted in urine [37], which allowed us to take several samples within the same animal, but more importantly to translate our findings to a clinical trial in premature neonates. We are the first to study the effect of LED phototherapy on premature neonates below 35 weeks gestational age, which constitute the most relevant patient population, since the vast majority of them receive phototherapy and their anti-oxidative capacities are the weakest [38]. Preterm neonates are more vulnerable to oxidative stress and receive more frequent and longer phototherapy than their term counterparts [38]. Performing clinical trials using blood markers in this patient group is always ethically challenging, since they already need regular blood

transfusions, because they cannot compensate for all the blood drawn for diagnostic testing. Obviously, drawing blood causes additional pain and discomfort or exposes them to an additional risk of infections via a central line. Using a urinary marker allowed us to collect samples before, during and after phototherapy and determine a postnatal course of the marker. This is relevant, since several oxidative stress markers, including 8-OHdG, increase postnatally, which needs to be taken into account when assessing phototherapy effects [39,40]. Chapter 5 describes the first longitudinal study on LED phototherapy effects analysed with generalized estimating equations modelling, which allowed us to correct for increasing postnatal age during phototherapy.

Our results show no genotoxic effects of LED phototherapy for irradiances up to 100 μW in rats and up to 35 μW/cm^2/nm in preterm infants and therefore mitigates concerns about LED phototherapy-induced oxidative DNA damage. Thereby, our data form the first step for the clinical evaluation of high irradiance LED phototherapy and alternative treatment regimes, including intermittent high irradiance phototherapy or two-sided body exposure. With regards to safety, more studies are needed in preterm infants to study the effects higher intensity treatment (>35 μW/cm^2/nm), longer treatment, and long term outcomes and side effects. The effect of phototherapy on mortality in ELBW infants will have to be reinvestigated using LED phototherapy. Furthermore, the recently reported epidemiological correlation between infantile cancer and phototherapy [41] will have also have to be assessed with LED phototherapy. Alternatively, potential carcinogenic effects could be tested in cancer-predisposed animal models (e.g. *p53* mutants) under controlled circumstances.

4. UDCA, OCA and intestinal bilirubin conjugation

Although phototherapy is highly effective, it cannot prevent neonatal hyperbilirubinemia and does not completely eliminate the need for exchange

transfusions. Also, patients with Crigler-Najjar type I, need on average 10-16h of phototherapy a day, which severely impairs their quality of life [42]. Therefore, there is a continuous search for alternative or complementary treatments. Such treatments could theoretically shorten hospital stay, minimize exposure to toxic bilirubin concentrations and decrease the need for exchange transfusions. This study identifies two therapeutic bile acids as potential new treatments for neonatal hyperbilirubinemia; UDCA and OCA. Whereas OCA is a relatively new player in the field [43], UDCA is already FDA-approved in preterm neonates and has even been shown to cause additional advantage when used in parallel to phototherapy in neonates[44]. In chapter 5 we show in *hUGT1*1* mice that both compounds not only decrease plasma UCB, but also brain UCB and thereby bilirubin neurotoxicity risk. These changes are accompanied by significant inductions of hUGT1A1. In Gunn rats, which are UGT1A1-deficient, OCA did not have any effect and the effect of UDCA was significantly smaller. Although a direct comparison between different species cannot be made, it does indicate that UDCA works via both UGT1A1-dependent and -independent mechanisms, whereas in OCA-mediated effects, UGT1A1 plays a pivotal role.

The majority of neonatal hyperbilirubinemia research has been performed in models such as the Gunn rat and *UGT1A1* knockout mouse. However, human neonates cannot be considered as completely UGT1A1 deficient; they have inducible UGT1A1 expression that can be enhanced to decrease plasma UCB levels[45]. This promising therapeutic mechanism has hardly been investigated, since it is impossible to assess in completely UGT1A1-deficient models. Even when UGT1A1 expression had been studied in wild-type or heterozygote rats, the translational potential remained questionable, since the UGT1A1 promoter and its transcription factor binding sites are not conserved between species [46]. The development of a humanized model that shows hyperbilirubinemia limited to the neonatal period, opened the door for UGT1A1 induction studies and a new therapeutic strategies in neonatal hyperbilirubinemia [47]. In the *hUGT1*1*

model, primarily intestinal, and not hepatic UGT1A1 was induced by UDCA and OCA. Although the contribution of intestinal UCB conjugation to bilirubin metabolism in human neonates has not (yet) been described, it has also never been investigated. Our findings in chapter 6 and work by others [47-49] highlight the potential important but yet underestimated role of intestinal UCB conjugation, as described in chapter 1.

To reveal the mechanism of UDCA and OCA-induced UGT1A1 expression, it would be appropriate to test both compounds in *Fxr* knockout mice in a *hUGT1*1* background. Also, since FXR transcriptionally regulates PXR [50], both compounds should be tested in *Pxr* knockout mice in a *hUGT1*1* background. Clinically, UDCA is already FDA-approved in neonates and has already been shown to have therapeutic advantage in a cohort of 80 term infants [44]. To follow up on this, a larger study will need to be conducted also including preterm infants. In addition, the preventative capacity of UDCA will need to be assessed, by starting treatment at birth or in the first 2 postnatal days. During such a study, UCBfree measurements and UCBfree/TSB ratio would need to be reported to predict bilirubin-induced neurotoxicity. For OCA, its safety and tolerance in children and neonates would first need to be established before efficacy testing could be ensued. In adults however, OCA is already FDA-approved and it could potentially be beneficial in patients with Crigler-Najjar type II. These patients have a low, but inducible level of UGT1A1, and a subset of them is now treated with phenobarbital, a Constitutive Androstane Receptor (CAR) agonist, which is also known to induce UGT1A1. Potentially, this patient population might benefit from an FXR-agonist, that, unlike phenobarbital, has no sedative side effects.

OCA acts systemically on several organs and therefore side effects could be expected [51]. Since the FXR-induced UGT1A1 induction seems to mainly work intestinally, we think it is worthwhile to test an intestine specific FXR-agonist,

i.e., feraxamine [52]. Feraxamine is a synthetic FXR agonist, that is hardly absorbed by the intestine and leads to minimal plasma concentrations when administered orally. Despite this, it is still able to induce the FXR-induced favourable metabolic profile in mice, including reduced obesity and insulin resistance and brown adipose tissue browning. It is therefore believed to mimic the intestinal FXR activation by bile acids after a meal [53].

5. UDCA and intestinal bilirubin metabolism

In addition to the *hUGT1*1* mice, we found that UDCA also significantly decreases plasma UCB in neonatal Gunn rats, independent of hUGT1A1. The importance of intestinal UCB handling in UDCA-action already became apparent in previous work by Cuperus et al.[54], in which adult Gunn rats were treated with UDCA. From this data, it was concluded that the UDCA-decreasing potential of UDCA could be explained for 20% by enhanced biliary excretion and for 80% by intestinal translocation, such as transmucosal excretion, decreased intestinal reabsorption or intestinal trapping. These findings, and numerous studies that successfully decreased plasma UCB by stimulating fecal excretion (Chapter 1), suggest that intestinal metabolism plays a pivotal, yet poorly described role in unconjugated bilirubin metabolism. In the following sections, we will discuss several hypothetical mechanisms that can potentially contribute to intestinal bilirubin elimination.

5.1 Intestinal bilirubin conjugation

We hypothesize that the intestine is an alternative conjugation site of blood-derived UCB. Like hepatocytes, enterocytes express UGT1A1. In addition, they express ABCC2 on their luminal and ABCC3 on their basolateral membrane, potentially facilitating export of CB from the cell. This transport will occur either towards the intestinal lumen by ABCC2 or back into the blood by ABCC3 (fig. 5A) . The latter is in agreement with the fact that intestinal UGT1A1 development coincides with intestinal ABCC3 expression in mice (fig. 5B) [55].

Subsequently, OATP1B1 and 1B3 can efficiently transport CB into the liver [56], after which it is excreted via the bile in the intestinal lumen. To test this hypothesis, the relative contribution of intestinal hUGT1A1 would have to be established, which requires *UGT1A1* knockout mice with tissue specific hUGT1A1 expression in liver, intestine or kidney. In the intestine-expressing *hUGT1A1*-mice, CB should be determined, although challenging, in intestinal tissue, blood, bile and feces. Presence of CB in plasma and bile indicates basolateral transport from the enterocyte into the blood, whereas the sole presence of CB in feces indicates intraluminal disposal. If ABCC3 were to be responsible for this transport, crossing intestine-specific *hUGT1A1*-mice with *Abcc3* knockout mice will result in complete absence of CB in plasma and a strong rise in plasma UCB with potentially death by kernicterus. On the other hand, if ABCC2 were responsible for intraluminal transport of CB, crossing with an *Abcc3* knockout would likewise result in a rise in plasma UCB. Alternatively, knocking out one of the ABC transporters might lead to compensation by the other, causing a shift of CB from plasma to feces or vice versa. As a first step, these experiments could be simulated *in vitro* using a transwell system with hUGT1A1-expressing enterocytes.

Finally, the postnatal intestinal hUGT1A1 expression in the *hUGT1*1* mice should be compared with the expression in human neonates, to establish the translational potential of this animal model. The latter is however practically almost impossible, since intestinal tissue cannot be obtained from healthy infants. As suboptimal alternative, intestinal cell material could be obtained from laparotomies or shedded intestinal cells [57].

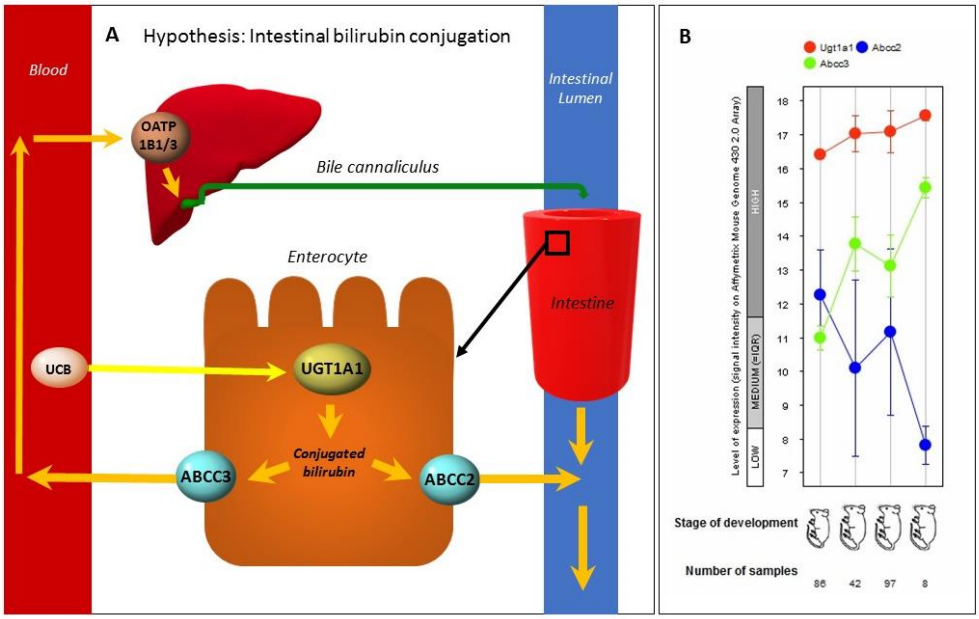

Figure 5: Schematic overview of hypothesized intestinal bilirubin conjugation and role of ABCC2 and ABCC3. A) *UCB is taken up by the enterocyte via a yet unknown mechanism. In the enterocyte UCB is conjugated by UGT1A1 and CB is subsequently either transported into the intestinal lumen by ABCC2 or into the blood by ABCC3. In the latter case, CB is transported into the liver by OATP1B1 and OATP1B3 and subsequently excreted in the intestine via the bile.* ***B)*** *Developmental course of gene expression of UGT1A1, ABCC2 and ABCC3 in intestine in mice, as derived from Genevestigator* [55]*. Stages of development represent postnatal day 4-15, 16-63, 64-255 and 255 to end of life.*

5.2 Transmucosal excretion or intestinal reabsorption

Several studies have shown net transmucosal UCB excretion from the blood into the intestinal lumen in severe unconjugated hyperbilirubinemia [54,58,59]. Also, especially in neonates, UCB is known to be subject to extensive intestinal reabsorption and thereby enterohepatic circulation [60]. It is unclear however whether these transmucosal UCB movements occur by simple diffusion or whether specific transporters are involved. Two potential luminal UCB transporters; MDR1 P-glycoprotein and OATP2B1, are expressed in intestine, but their contributions to intestinal bilirubin metabolism have not been identified [61,62]. Experiments using *MDR1* or *OATP2B1* knockouts in both *in vitro*

transwell-systems or in hyperbilirubinmic animal models would provide clarity on the intestinal role of these transporters. Alternatively, intestinal permeability is increased under hyperbilirubinemic circumstances, which could also play a possible role. To test this, intestinal permeability inhibitors and enhancers should be tested in animal models of hyperbilirubinemia and their effect on plasma and fecal UCB and fecal urobilinoids should be established.

6. Conclusions and future perspectives

Unconjugated neonatal hyperbilirubinemia is commonly seen in (preterm) infants and may result in permanent brain damage. Nevertheless, its pathogenesis is complex and still not fully elucidated. Although our understanding of this disease has vastly increased over the years, the translation of this knowledge to clinical care and new treatments has been limited. The success of phototherapy and the lack of accurate tools to quantify bilirubin-induced neurotoxicity has created an attitude of satisfaction and relaxation towards the current neonatal hyperbilirubinemia management, whereas improvements can certainly be made.

Current management guidelines are based on TSB levels, whereas TSB is a poor predictor of bilirubin-induced neurotoxicity. Instead, UCBfree and the UCBfree/TSB ratio are better predictors, but not routinely used in clinic. In this thesis, we provide the first step towards UCBfree-based treatment guidelines by reporting the postnatal course of these diagnostic markers. Furthermore, although phototherapy is very effective, the currently used irradiances and durations are still based on the limited light irradiances of conventional FT phototherapy devices and their (most probably heat-induced) side effects. With the introduction of LED phototherapy, it is possible to administered higher irradiance phototherapy without significant heat production, but this irradiance is not routinely used in the clinic. The results in this thesis attenuates

concerns about potential detrimental side effects regarding oxidative DNA damage of LED phototherapy and opens the door for more elaborate testing of high-irradiance LED phototherapy and alternative treatment protocols. Alternative phototherapy regimens could potentially shorten the phototherapy duration, which is currently often several days and severely interferes with the mother-child interaction.

Another way to minimize treatment duration, would be to prevent the rise of neonatal UCB or use alternative therapies in adjunct to phototherapy. Since >80% of preterm neonates currently receive phototherapy [63,64], we belief that a preventative therapy, in analogy to prenatal corticosteroids for the prevention of infant respiratory distress syndrome (IRDS), would be valuable and decrease the need for phototherapy. In this thesis, we show that the two therapeutic bile acids; UDCA and OCA, effectively decrease UCB in brain and plasma in an mouse model for neonatal jaundice. Especially the use of UDCA seems rather easily translatable to the clinic, since it is already FDA-approved for neonatal use and its therapeutic effect in infants has already been shown when used in parallel to phototherapy [44]. Finally, in this thesis we emphasize the importance of intestinal bilirubin metabolism and its potentially underestimated role in neonatal hyperbilirubinemia. Further research should elucidate the exact mechanisms of intestinal bilirubin handling. Unravelling these mechanisms could open up an array of new therapeutic strategies for unconjugated hyperbilirubinemia, for both neonates and Crigler-Najjar patients.

Although the “yellow brick road” of hyperbilirubinemia research has been walked by many researchers, with this thesis we believe to have extended the road a bit further towards better understanding and treatment of neonatal hyperbilirubinemia and eventually better care for preterm jaundiced neonates.

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