Appendices

Summary, samenvatting, dankwoord, list of abbreviations, biografie, list of publications

Summary

In this thesis we developed and refined treatment strategies for severe unconjugated hyperbilirubinemia in the Gunn rat model. Severe unconjugated hyperbilirubinemia, as occurs in Crigler-Najjar disease and neonatal hemolytic jaundice, is associated with brain damage and death. Routine treatment mainly consists of phototherapy, which converts bilirubin into more readily excretable photo-isomers. Although generally effective, phototherapy has some disadvantages. The lifelong phototherapy in Crigler-Najjar patients, who suffer from inherited jaundice, may last up to 16h per day but fails to prevent brain damage in ~25% of these patients. Neonatal phototherapy, although mostly efficient and safe, sometimes fails to achieve a sufficient decrease in plasma bilirubin levels. This may, ultimately, also lead to permanent brain damage in these patients.

These considerations demonstrate the need for alternative treatment strategies. Most hypobilirubinemic treatments, as described in **chapters 1** and **2**, stimulate the fecal excretion of bilirubin *via* the bile. Biliary bilirubin excretion, however, is highly inefficient in patients with Crigler-Najjar disease and, to a lesser extent, in patients with neonatal jaundice. This is due to an inactivated (Crigler-Najjar disease) or immature (neonatal jaundice) isoform of the enzyme UDP-glucuronosyltransferase. This hepatic enzyme catalyzes the transfer of glucuronic acid to bilirubin, thus forming bilirubin monoglucuronoside or diglucuronoside. This so-called conjugated bilirubin is more water soluble, and can readily be excreted into the bile. Bilirubin, however, is not exclusively excreted *via* the bile, but may also enter the intestinal lumen *via* direct transmucosal excretion from the blood. The efficiency of this excretory pathway is decreased, however, by reabsorption of unconjugated bilirubin from the intestinal lumen. This reabsorption can be prevented by intestinal capture; the binding of intestinal unconjugated bilirubin to orally administered agents.

In **chapter 3** we decided to assess whether oral treatment with the bile salt ursodeoxycholate (UDCA) would enhance the transmucosal excretion of bilirubin. We chose UDCA because it binds bilirubin *in vitro*, and because it is safely used in the treatment of several biliary diseases. To study bilirubin fluxes, we decided to use radioactively labeled bilirubin, which necessitated the use of an animal model. We consequently assessed the effects of UDCA feeding in Gunn rats, the well-established animal model for unconjugated hyperbilirubinemia. This approach allowed us to demonstrate that UDCA decreases plasma bilirubin levels and enhances its transmucosal excretory pathway. UDCA treatment did not only decrease bilirubin in the circulation, but also lowered the amount of bilirubin in

the tissues. Taken together, these data support the feasibility of UDCA treatment in hyperbilirubinemic patients.

Since chapter 3 demonstrated the importance of transmucosal excretion, we continued our pursuit to enhance this pathway in **chapter 4**. This chapter evaluated whether acceleration of the gastrointestinal transit by the laxative polyethylene glycol could treat unconjugated hyperbilirubinemia in Gunn rats. Clinical conditions that delay the gastrointestinal transit, such as fasting, seem to increase plasma bilirubin levels. Conditions that accelerate the gastrointestinal transit, such as frequent feedings in the neonatal period, are associated with a decrease in plasma bilirubin. Acceleration of the gastrointestinal transit could, theoretically, lower the intestinal bilirubin concentration. A lower intestinal bilirubin concentration, in turn, could create a diffusional bilirubin shift from the blood into the intestinal lumen. None of the above-described conditions, however, had directly linked the transit time to plasma bilirubin levels. We investigated this link by treating Gunn rats with the laxative polyethylene glycol, or with loperamide (which delays transit). We demonstrated that polyethylene glycol treatment *accelerated* the gastrointestinal transit and, simultaneously, *decreased* plasma bilirubin levels. This decrease did not originate from an increased biliary disposal of bilirubin. We thus concluded, by inference, that acceleration of the gastrointestinal transit must enhance the transmucosal excretion of bilirubin, since this is the only alternative excretory pathway that allows bilirubin to enter the intestinal lumen. Loperamide treatment, in contrast, *delayed* the gastrointestinal transit and thereby *increased* plasma bilirubin levels.

Our approach demonstrated, for the first time, a linear relationship between the gastrointestinal transit time and bilirubin levels in Gunn rats. The strength of this relationship implicated that pharmacological manipulation of the gastrointestinal transit may well be used to treat unconjugated hyperbilirubinemia. Because polyethylene glycol is a safe and well-tolerated treatment for constipation, we feel that the results in this chapter merit a randomized clinical trial in hyperbilirubinemic Crigler-Najjar patients.

In **chapter 5** we investigated, in Gunn rats, whether known hypobilirubinemic treatments (e.g. phototherapy and/or oral capture agents) exert their therapeutic effect by acceleration of the gastrointestinal transit. A review of the literature had shown us that each of these treatments was troubled with side effects, mostly diarrhea, that did affect the gastrointestinal transit. In chapter 5, however, we clearly demonstrated that each of the aforementioned treatments decreased plasma bilirubin without affecting the gastrointestinal transit. We consequently evaluated whether additional treatment with polyethylene glycol could increase the efficacy of these treatments. This experiment demonstrated that phototherapy combined with polyethylene glycol decreased the plasma bilirubin levels in Gunn

rats by almost 70%. These results identified polyethylene and phototherapy as the most potent hypobilirubinemic treatment combination that has been tested so far in the Gunn rat model.

Eventually, all therapy should prevent neurotoxicity by decreasing tissue rather than plasma bilirubin concentrations. UCB_{free}, the small fraction of unconjugated bilirubin that is not bound to plasma albumin, is able to diffuse from the plasma into the tissue pool. Decreasing this fraction, for instance by increasing its binding to albumin, would thus be expected to decrease tissue bilirubin levels. In **chapter 6** we investigated the effect of phototherapy and phototherapy + albumin infusion on UCB_{free} and brain bilirubin levels during permanent and acute hemolytic jaundice in Gunn rats. Our results demonstrated a synergistic effect of albumin treatment to phototherapy in Gunn rats. In non-hemolytic Gunn rats, used as a model for Crigler-Najjar disease, we showed that albumin treatment enhanced the phototherapy-induced decrease in plasma UCB_{free} and brain bilirubin levels by 32-25%. Phototherapy + albumin completely prevented bilirubin accumulation in the brains of hemolytic Gunn rats, used as a model for acute neonatal jaundice. Interestingly, phototherapy alone failed to prevent bilirubin accumulation in the brains of these animals. Our findings, therefore, do not only demonstrate the efficacy of adjunct albumin treatment during phototherapy in Gunn rats, but also question the therapeutic effects of routine phototherapy. UCB_{free} correlated well with the concentrations of bilirubin in the brain, underlining its potential use as predictor for bilirubin damage in clinical practice. Taken together, our results underline the need for a large-scale clinical trial to evaluate the use of adjunct albumin in phototherapy-treated Crigler-Najjar and neonatal patients.

In the final chapter of this thesis, **chapter 7**, we place our results in a experimental and clinical framework, and discuss future perspectives.

In this thesis we have developed and refined treatment strategies for unconjugated hyperbilirubinemia in the Gunn rat. These strategies may serve as an alternative for routine treatment, and may well prevent bilirubin-induced brain damage in hyperbilirubinemic patients. We consequently feel that our results can be used as a framework for the development of clinical controlled trials in Crigler-Najjar patients and in severely jaundiced neonates.