Gene therapy with adeno-associated viral vectors for inherited hyperbilirubinemia: towards a clinical trial for Crigler-Najjar syndrome

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Summary

Crigler-Najjar syndrome type I (CNI) is a form of familial severe unconjugated hyperbilirubinemia, caused by UDP-glucuronosyltransferase 1A1 (UGT1A1) deficiency. Presently these patients are treated with daily (8-14h/day) phototherapy that highly affects their quality of life. This treatment becomes ineffective at later age and most CNI patients do need a liver transplant. The limited availability of donor organs and the adverse effects of life-long immune suppression render a liver directed gene therapy a preferable option. In the relevant animal model for CNI, the Gunn rat, our group showed that single-stranded AAV vector serotype 1 (ssAAV1) liver-directed gene therapy seems also feasible for CN syndrome. In Chapter II we show that this single-stranded vector is only effective in male rats. Such a gender difference will limit the clinical use of an AAV vector when aiming to treat an autosomal disorder like CN syndrome. This gender difference in ssAAV-mediated liver transduction efficiency appeared to be mainly caused by the rate limiting conversion of single stranded genome into an active double stranded genome in female livers. Subsequently we demonstrated that $3 \times 10^{11}$gc/kg of double-stranded or self-complementary (scAAV) vector provides therapeutic correction both in male and female rats, a dose shown recently to be safe and effective in patients upon systemic injection to treat hemophilia B. The developed scAAV vector efficient in both genders seems suitable for treating of CN and provides a gene therapy platform to treat other autosomal inherited liver disorders.

In recent AAV-mediated liver-directed gene therapy clinical trials the patients developed an immune response against the viral vector. In order to reduce the risk of a deleterious immune response, development of a rational transient immune suppression regimen seems essential. In Chapter III we investigated if immune suppression can be used to block immune responses that could prevent sustained correction in patients. We showed that transient Mycophenolate Mofetil (MMF) treatment reduces immune responses towards AAV in the Gunn rat. However, at clinical relevant doses this drug compromises the efficacy of ssAAV transduction *in vitro* and *in vivo*. Subsequently we showed *in vitro* that this was caused by depletion of the intra-cellular guanosine pool, that impaired DNA synthesis needed to convert single-stranded AAV genomes into an active double stranded DNA. Consequently, in contrast to ssAAV, the efficacy of scAAV is not reduced by MMF. This study is very relevant for the translation of a ssAAV-mediated liver directed gene therapy.
To reduce the risk of a deleterious immune response, in addition to the use of immune suppressive drugs, vector dose reduction seems essential. In this respect the use of different serotypes and the use of the more effective double stranded scAAV hold promise. We have shown that a portal vein injection AAV serotype 1 provided efficient correction of serum bilirubin levels in the Gunn rat. Recently others reported that serotype 5 and especially 8 transduce hepatocytes very efficiently in mice and non-human primates. Therefore in Chapter IV we compared the efficacy of scAAV serotypes 1, 5 and 8 in the Gunn rat upon portal vein administration. In this chapter we demonstrate that scAAV8 and scAAV1 are very effective in the Gunn rat. Surprisingly, no correction of serum bilirubin was seen upon administration of scAAV5 irrespective of the route of administration. To further investigate this we compared its efficacy in different rodent models. Which confirmed that the liver transduction efficiency of scAAV5 in rats is very inefficient compared to mice. Recently, others reported that in a rabbit model liver transduction of scAAV5 is also very poor. The mechanism behind this large variation in liver transduction efficacy of scAAV5 between animal species is unknown. In any case it does not allow proof of concept of this potentially interesting serotype, in the relevant animal model for CN syndrome the Gunn rat.

Although some integration into the host genome does occur, studies with recombinant AAV demonstrated that it mainly persists in an episomal form in vivo. Since most studies have been performed in proliferating liver with ssAAV, knowledge about integration of scAAV in quiescent liver is very limited. Since host genome integration is very relevant for long-term safety of scAAV we investigated this in Chapter V in the Gunn rat, an animal model in which the liver is quiescent and liver architecture is normal. Injection of scAAV provides sustained correction of serum bilirubin in this animal model. Upon a two-thirds partial hepatectomy at 12 months post vector administration, this correction was largely retained. In contrast, after a partial hepatectomy at 3 months it was lost completely. The difference in persistence between both time points was confirmed by the vector genomic copy numbers in liver and presence of clusters of UGT1A1 expressing hepatocytes. LAM-PCR analysis showed that scAAV integration, like reported for ssAAV earlier, is very low also after a year. The number of integrants was too low to demonstrate a significant increase in time, underscoring the excellent safety of scAAV in this respect.
Presently, AAV-mediated gene therapy is the most promising approach for the treatment of monogenic liver disorders. An important limitation of this vector however is its small cloning capacity. For instance the human factor VIII cDNA is too large to be efficiently encapsulated in an AAV vector, rendering this vector unsuitable for the treatment of hemophilia A. Moreover, although a single treatment of a patient suffering from severe hemophilia B resulted in FIX production for 12 months, the longevity of scAAV mediated liver directed gene therapy is still unknown. Therefore re-treatment may be needed at some point. Considering this, the development of an additional viral vector that is safe and efficient seems necessary. The liver tropism of adenovirus renders it an interesting option in this respect. In Chapter VI we demonstrated that careful designed second generation adenoviral vectors did provide life-long correction of hyperbilirubinemia in the Gunn rat. From the vectors tested, the most efficient vector was an adenoviral – AAV hybrid vector, in which the liver specific expression cassette was flanked by the ITR’s from AAV2. This increased efficacy appeared due to enhanced expression of the encoded UGT1A1 mRNA, which may be caused by the transcriptional activity of the AAV-ITR. In addition, the therapeutic dose of this vector seemed low enough to warrant safety upon systemic injection, suggesting it may be suitable in a clinical setting.

Our aim is to treat CN patients in the Netherlands that need phototherapy to prevent bilirubin encephalopathy. To be able to demonstrate therapeutic efficacy in the small group of patients eligible for this treatment, pre- and post- treatment pathophysiology have to be evaluated extensively. In Chapter VII we studied the serum bilirubin levels and presence of bilirubin in bile in these patients. In addition we determined the correlation between bilirubin glucuronides in bile and the enzymatic activity restored by gene therapy in the Gunn rat. This showed that the presence of bilirubin glucuronides in bile is a sensitive marker for hepatic UGT1A1 activity. However, the invasive procedure of bile sampling and the effect of phototherapy on the presence of unconjugated bilirubin in bile render it not the ideal marker. Therefore, we investigated the use of additional UGT1A1 substrates as potential serum parameters for hepatic UGT1A1 activity. These studies revealed that the cholesterol lowering drug ezetimibe may potentially be suitable as such parameter.