Helper-dependent adenoviral vectors for liver-directed gene therapy

Nicola Brunetti-Pierri$^{1,2}$ and Philip Ng$^{3,*}$

$^{1}$Telethon Institute of Genetics and Medicine, Naples 80131, Italy, $^{2}$Department of Pediatrics, Federico II University of Naples, Naples 80131 Italy and $^{3}$Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, 630E, Houston, TX 77030, USA

Received December 22, 2010; Revised and Accepted March 28, 2011

Helper-dependent adenoviral (HDAd) vectors devoid of all viral-coding sequences are promising non-integrating vectors for liver-directed gene therapy because they have a large cloning capacity, can efficiently transduce a wide variety of cell types from various species independent of the cell cycle and can result in long-term transgene expression without chronic toxicity. The main obstacle preventing clinical applications of HDAd for liver-directed gene therapy is the host innate inflammatory response against the vector capsid proteins that occurs shortly after intravascular vector administration resulting in acute toxicity, the severity of which is dependent on vector dose. Intense efforts have been focused on elucidating the factors involved in this acute response and various strategies have been investigated to improve the therapeutic index of HDAd vectors. These strategies have yielded encouraging results with the potential for clinical translation.

INTRODUCTION

Helper-dependent adenoviral (HDAd) vectors are deleted of all viral-coding sequences, can efficiently transduce a wide variety of cell types from various species independent of the cell cycle and can result in long-term transgene expression. Several small and large animal models of genetic disorders can be corrected effectively and for long term by HDAd without chronic toxicity (1). An important advantage of HDAd vectors is their large cloning capacity of up to ~37 kb, which allows for the delivery of large therapeutic genes and even whole genomic loci, multiple transgenes and large cis-acting elements to enhance, prolong and regulate transgene expression. The HDAd vector genome remains episomal in the nuclei of transduced cells, where it associates with cellular histones and, depending on the nature of the stuffer sequence, may undergo repression or can be maintained transcriptionally active (2). Because of its non-integrating nature, HDAd vectors are not associated with an increased risk of insertional carcinogenesis (3). Although clearly superior to early-generation Ad vectors in terms of safety and efficacy, clinical applications of HDAd have been hampered by an acute toxic response mediated by the vector capsid proteins in a dose-dependent manner. This review will focus on the current progress towards the clinical applications of HDAd vectors for liver-directed gene therapy, highlighting particularly interesting in vivo studies and discussing the important obstacles preventing clinical translation and strategies that have recently been developed to overcome them.

LIVER-DIRECTED GENE THERAPY

The liver is a very attractive target for gene therapy because it is a central organ in many metabolic processes and several inherited metabolic disorders have their origin in the liver (Table 1). Therefore, the hepatocyte is a key target for gene transfer directed at the correction of inborn errors of metabolism and hemophilias. The majority of the preclinical studies for in vivo liver-directed gene therapy, performed in various disease animal models, have demonstrated that HDAd can lead to long-term, sometimes lifelong, phenotypic correction in the absence of chronic toxicity, supporting the potential of HDAd for clinical applications (4,5). Importantly, long-term expression by HDAd has also been recapitulated in clinically relevant large animal models, including dogs and non-human primates (6–10). These results indicate that

*To whom correspondence should be addressed. Tel: +1 7137984158; Fax: +1 7137987773; Email: png@bcm.edu

© The Author 2011. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com
was detected (15). Also unfortunate is that this study has yet
infusion. Unfortunately, no evidence of FVIII expression
thy, but all these values returned to baseline by day 19 post-
marked increase in interleukin-6 (IL-6), thrombocytopenia
patient (15). This subject developed grade 3 liver toxicity,
transduction does not, at least alone, appear to necessarily
dose-dependent and preferential, high efficiency hepatocyte
applications of HDAd vectors, the severity of this response
INNATE INFLAMMATORY RESPONSE

Diabetes Type 1 diabetes Mouse Islet neogenesis and reverse of diabetes (83)
Type 2 diabetes Mouse Improved glucose homeostasis (84)

“This study includes a single human patient.

Hemophilias are superior to first-generation adenoviral (FGAd) vectors in terms of efficacy and chronic toxicity. With FGAd, the expression of viral genes from the vector backbone in fact is directly cytotoxic and also provokes an adaptive cellular immune response against the transduced cells resulting in transient transgene expression and chronic toxicity. This type of toxicity does not occur with HDAd vectors because they are devoid of all viral-coding sequences. However, the viral capsid, which is identical for both FGAd and HDAd, is responsible for triggering an acute inflammatory response in a dose-dependent manner (11,12). This capsid-mediated acute toxicity is lethal in non-human primates following systemic intravascular injection of either FGAd or HDAd at doses \( \geq 1 \times 10^{11} \) viral particles (VP)/kg (11,13,14). However, while FGAd vectors also result in a late phase of toxicity, occurring days to weeks after vector injection owing to low-grade expression of viral proteins from the vector backbone, the toxic response elicited by HDAd appears to be restricted exclusively to the first 24–48 h post-vector administration owing to the absence of viral gene expression. There has been a single case of intravascular administration of HDAd into a human patient. In this clinical trial, \( 4.3 \times 10^{11} \) VP/kg of a HDAd-expressing factor VIII (FVIII) was intravenously injected into a hemophilia A patient (15). This subject developed grade 3 liver toxicity, marked increase in interleukin-6 (IL-6), thrombocytopenia and laboratory signs of disseminated intravascular coagulopathy, but all these values returned to baseline by day 19 post-infusion. Unfortunately, no evidence of FVIII expression was detected (15). Also unfortunate is that this study has yet to be published in a peer-reviewed format so that much of the details remain unknown.

INNATE INFLAMMATORY RESPONSE

Although the acute toxicity is clearly an obstacle for clinical applications of HDAd vectors, the severity of this response is dose-dependent and preferential, high efficiency hepatocyte transduction does not, at least alone, appear to necessarily cause a potent innate inflammatory response in mice (16). Therefore, major efforts have been focused in understanding the biology of the acute toxic response with the goal of developing strategies to block it or to improve the vector’s ability at transducing hepatocytes so that lower, non-toxic doses can be administered.

Understanding the causes of the toxic response elicited by systemic injection of Ad-based vectors turned out to be a difficult task because multiple factors are involved. Within the bloodstream, the Ad comes into contact with the blood cells and several blood-borne proteins, all of which play a role in vector biodistribution and acute toxicity. In the blood, Ad binds to factor X (FX), as well as other vitamin K-dependent serine proteases, such as factors VII, IX and protein C (17–19), and efficiently target low-density lipoprotein receptor-related protein and heparan sulfate proteoglycans on hepatocytes that facilitate virus entry (19,20). The Ad particles activate the complement system through binding and activation of proteins in the classical and alternative complement pathways (21). Interactions of Ad with complement, including C3 and C4BP (22–24), result in the adhesion and migration of infiltrating leukocytes and platelet aggregation, and high levels of proinflammatory cytokines and chemokines (22).

Human plasma components (notably Ad type-specific antibodies), preventing the re-administration of the same vector serotype, play a role in the acute toxicity. Both neutralizing anti-Ad antibodies (22,25) and non-neutralizing or naturally occurring (non-specific cross-reacting) antibodies (22,26,27) may contribute to the acute toxicity by opsonizing the viral particles and rendering them more susceptible to Fc-mediated uptake by macrophages, which in turn become activated to secrete proinflammatory cytokines. This model is supported by studies showing that pre-existing immunity to Ad is associated with increased mortality shortly after the systemic administration of Ad in mice (28) and significantly higher IL-6 levels in non-human primates (25).

The clinical features of the acute toxic response observed in non-human primates resemble the shock septic reaction with hypotension, hemodilution, diffuse drug and organ dysfunction.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Species</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilias Hemophilia A</td>
<td>Mouse</td>
<td>Long-term expression or anti-hFVIII antibody response; tolerance in newborn-injected mice</td>
<td>(62–65)</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>Long-term correction</td>
<td>(66,67)</td>
</tr>
<tr>
<td></td>
<td>Human*</td>
<td>No evidence of FVIII expression; toxic response</td>
<td>(15)</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Mouse</td>
<td>Long-term correction</td>
<td>(68,69)</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>Long-term correction</td>
<td>(10,70)</td>
</tr>
<tr>
<td>Inborn errors of metabolism</td>
<td>Mouse</td>
<td>Long-term correction</td>
<td>(71,72)</td>
</tr>
<tr>
<td>Ornithine transcarbamylase deficiency</td>
<td>Mouse</td>
<td>Short-term correction in newborn mice</td>
<td>(73)</td>
</tr>
<tr>
<td>Arginase deficiency</td>
<td>Mouse</td>
<td>Increased survival</td>
<td>(74)</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>Mouse</td>
<td>Long-term correction</td>
<td>(75)</td>
</tr>
<tr>
<td>Glycogen storage disease 1a</td>
<td>Rat</td>
<td>Long-term correction</td>
<td>(5,76)</td>
</tr>
<tr>
<td>Crigler-Najjar syndrome</td>
<td>Mouse</td>
<td>Long-term correction</td>
<td>(77)</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>Mouse</td>
<td>Long-term correction</td>
<td>(47,80)</td>
</tr>
<tr>
<td>ApoE deficiency</td>
<td>Mouse</td>
<td>Long-term correction</td>
<td>(79–81)</td>
</tr>
<tr>
<td>Familial Hypercholesterolemia</td>
<td>Mouse</td>
<td>Long-term correction</td>
<td>(82)</td>
</tr>
<tr>
<td>ApoA1 deficiency hypoalphalipoproteinemia</td>
<td>Mouse</td>
<td>Islet neogenesis and reverse of diabetes</td>
<td>(83)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Mouse</td>
<td>Improved glucose homeostasis</td>
<td>(84)</td>
</tr>
</tbody>
</table>
vasocongestion (11). In rodents, this reaction is dependent on reticuloendothelial system (RES)-derived platelet-activating factor (PAF), a lipid signaling molecule that is a known shock inducer. Interestingly, systemic intravenous injection of Ad stimulates the RES to upregulate PAF within minutes, consistent with the rapid onset of the toxic reactions observed in large animal models (29).

Intravascular delivery of Ad vectors induces an innate immune response very rapidly, which results in the activation of NF-κB pathway and transcription of host chemokine and cytokine genes (30). This innate immune response is activated by the interaction of Ad particles with Toll-like receptors (TLRs), the crucial components in pathogen recognition processes (31–37). TLRs have been implicated in Ad recognition at the level of the plasma membrane where TLR2 interacts with the Ad capsid proteins (38) and at the endosomal level where TLR9 interacts with the vector genome (32,36,37).

Internalized vector double-stranded DNA recognition occurs also via a TLR-independent nucleic acid-sensing mechanism (39), which is dependent on NALP3 and ASC, components of the innate cytosolic molecular complex known as the inflammasome (40).

The innate inflammatory response to Ad is clearly complex and multifactorial. However, a simple transient pre-treatment with the synthetic anti-inflammatory glucocorticoid Dexamethasone (DEX) prior to intravascular administration of Ad greatly reduced the associated innate toxicity in mice (41). Transient DEX pre-treatment also decreased the induction of neutralizing anti-Ad antibodies. Importantly, DEX pre-treatment did not reduce the efficacy of hepatocyte transduction by Ad.

**BARRIERS TO HEPATOCYTE TRANSDUCTION**

Within the liver, there are two major cell types that play an important role in transduction efficiency and acute toxicity: endothelial cells and Kupffer cells. Endothelial cells of the liver have a unique characteristic, i.e. the presence of fenestrations that allow small blood-borne particles to cross the vascular space to get in close contact with the hepatocytes. The liver fenestrations also form a structural barrier preventing large circulating macromolecules from accessing the hepatocytes. Several studies suggest that the size (~100 nm) and the number of the endothelial fenestrations of the liver play a key role in the efficiency of Ad-mediated hepatocyte transduction (Ad virion ≥ 100 nm) (16,42,43). Specifically, it was demonstrated that there is a positive correlation between the size of the fenestrations and the efficiency of hepatocyte transduction following systemic administration of Ad vectors (42,43). Fenestration size varies significantly among species in number and size (44) and these differences may also contribute to differences in hepatocyte transduction efficiency among species and possibly among subjects within the same species. Liver fibrosis and cirrhosis can lead to a decreased number of fenestrations and capillarization of normal sinusoids, resulting in changes of Ad vector biodistribution (45,46).

Kupffer cells of the liver avidly remove intravenously injected Ad vectors from the circulation predominantly through binding with scavenger receptors (47). In contrast to hepatocytes, uptake of Ad vectors by Kupffer cells is independent from the binding with FX (47,48). Ad uptake by the Kupffer cells reduces the efficiency of hepatic transduction, and is responsible for a nonlinear dose response (26,49,50). The relative contribution of Kupffer cells to Ad vector clearance may be dependent on the specific genetic context. For example, viral vector uptake in non-parenchymal liver cells in BALB/c mice is nearly 6-fold higher than in C57BL/6 mice. This difference in vector scavenging between mouse strains results in the approximately 3-fold higher transgene expression levels in C57BL/6 mice when compared with BALB/c mice (51). Similar variations may explain the well-known differences in hepatocyte transduction efficiency among different species.

Kupffer cells have also been implicated in the pathogenesis of the acute innate inflammatory response at least in mice. They are rapidly killed following the uptake of Ad but this does not result in elevated serum IL-6 (52,53). It is also interesting to note that systemic injection of low vector doses into mice and non-human primates resulting in low-efficiency hepatocyte transduction almost certainly results in substantial Ad uptake by Kupffer cells as they are the barrier responsible for the threshold effect to efficient hepatocyte transduction (26,27). Yet, these animals exhibit little, if any, manifestations of acute toxicity. Indeed, it has been suggested that Kupffer cells may, in fact, play a protective role (54).

In summary, Kupffer cells of the liver remove a significant proportion of injected vector, thus reducing the amount of vector available for hepatocyte transduction. Possible strategies currently under investigation to overcome the Kupffer cell barrier are based on modified Ad vector particles capable of evading Kupffer cell uptake (55). These vectors can transduce hepatocytes with higher efficiency and have potential for improving the therapeutic index of the vector (55). Alternative strategies of ‘masking’ the viral capsid have also been reported to attenuate the severity of the innate inflammatory response. Systemic administration of PEGylated Ad into mice resulted in a reduction in serum IL-6 compared with unPEGylated vector, but it also reduced hepatic transduction efficiency in non-human primates (56–58). Therefore, there is uncertainty regarding the real clinical potential of this approach.

Another barrier to hepatocyte transduction by Ad vectors appears to be erythrocytes, at least in humans. Over 90% of a typical dose of Ad vectors binds to and is neutralized by human erythrocytes ex vivo (59). Detail studies showed that erythrocytes from humans, but not from mice or rhesus macaques, bear the Ad receptor Coxsackie and Adenovirus Receptor (CAR) responsible for the sequestration of the vector (60). Furthermore, human erythrocytes, but not mice, bear the complement receptor 1 (CR1) that binds Ad in the presence of antibodies and complements. These results highlight the important potential limitations of animal models. A strategy to overcome sequestration by human erythrocytes is to coat the Ad vector with polymers containing quaternary amines (61).

Other strategies to improve the therapeutic index of the vector are based on physical approaches aimed at achieving preferential hepatocyte transduction. One such strategy was
to deliver the HDAd into the surgically isolated liver, thus limiting systemic exposure (7). This approach resulted in preferential hepatocyte transduction compared with systemic injection, no chronic toxicity and long-term, stable transgene expression for several years, post-vector administration in non-human primates. More recently, a minimally invasive, percutaneous balloon occlusion catheter-based method was reported to achieve preferential hepatocyte transduction, resulting in high level, stable transgene expression in non-human primates using clinically relevant low doses of HDAd (6,9). In this method, a sausage-shaped balloon is inflated in the inferior vena cava to occlude the hepatic venous outflow and the HDAd is injected directly into the liver via the hepatic artery (Fig. 1A). This novel method of vector delivery resulted in up to 80-fold higher level of transgene expression compared with peripheral intravenous injection of vector, and transgene expression persisted at high levels for at least 2.5 years. Adapted from Brunetti-Pierri et al. (9). IVC, inferior vena cava; HV, hepatic veins; HA, hepatic artery; PV, portal vein; bAFP, baboon alpha-fetoprotein; IV, intravenous.

CONCLUSIONS

The main obstacle preventing clinical applications of HDAd for liver-directed gene therapy is the host innate inflammatory response against the vector capsid proteins that occurs shortly after intravascular vector administration. This response is multifactorial and its mechanism(s) remains only partially understood. Clearly, a better understanding of this acute response is necessary for the development of novel strategies to overcome it. However, as the severity of this innate inflammatory response is dependent on the vector dose, strategies to overcome the barrier to efficient hepatocyte transduction so that sub-toxic amount of vector could be administered would also probably be beneficial in minimizing acute toxicity.

Conflict of Interest statement. None declared.

FUNDING

N.B.P. is supported by Fondazione Telethon (Rome, Italy) (TCBP37TELC). P.N. is supported by the National Institutes of Health (R01 DK069369 and R01 HL083047).

REFERENCES


