Successful liver-directed gene delivery by ERCP-guided hydrodynamic injection (with videos)

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Background and Aims: A simple, safe, targeted, and efficient in vivo DNA delivery system is necessary for clinical-grade liver-targeted gene therapy in humans. Intravascular hydrodynamic gene delivery has been investigated in large animal models, but translation to humans has been hampered by its technical challenges, invasiveness, and potential for significant cardiovascular adverse events. We posited that intrabiliary delivery of DNA plasmids via ERCP-guided hydrodynamic injection could overcome these obstacles.

Methods: Twelve pigs (40-50 kg) were divided into 3 groups (4 per group) and survived 21, 30, or 60 days. ERCP was performed by inflating a balloon catheter in the common hepatic duct and creating a closed space between it and the liver parenchyma. Last, a solution composed of plasmid/sleeping beauty (SB) mix was injected under pressure through the catheter into the closed space. Swine were killed at the 3 different time points and liver tissue harvested. Plasmid DNA expression and functional translated protein expression were assessed.

Results: ERCP-guided hydrodynamic delivery of naked plasmid DNA facilitated by pCytomegalovirus-Sleep Beauty (pCMV-SB) transposons was technically feasible and devoid of cardiovascular and local adverse events in all 12 pigs. Furthermore, plasmid DNA (both single and combination) was successfully transferred into swine hepatocytes in all 12 pigs. Additionally, stable integration of the DNA constructs in hepatocyte genomic DNA was reliably noted at all 3 time points. In the 4 swine that were kept alive to 60 days, successful genomic integration and subsequent protein expression was observed in the targeted liver tissue.

Conclusions: ERCP-guided hydrodynamic delivery of gene therapy may usher in the next chapter in gene therapy with the potential to impact a variety of single-gene, complex genetic, and epigenetic liver diseases. It also raises the possibility that other nucleic acid therapeutics (microRNA, lncRNA, siRNA, shRNA) could similarly be delivered.

(footnotes appear on last page of article)

The liver is affected in many acquired and inherited gene disorders. Devastating single-gene disorders, such as α_1-antitrypsin deficiency, cystic fibrosis, and many others, could theoretically be treated by inserting a corrected copy of the defective gene into affected liver cells. This presents an opportunity for the application of liver-targeted gene therapy, where the replacement of a single gene has been shown to have a significant clinical impact.[1] However, a technically simple, free of fatal adverse events, liver-specific method to deliver gene therapy does not currently exist. The lack of such a method is a major drawback to the effective treatment of millions of patients, many of whom are children.[2] Previous attempts at treatment of some of these disorders highlighted the potentially catastrophic side effects associated with the delivery vehicle as well as with the method of delivery.[3,4] Therefore, a critical but elusive step is the development of a clinical-grade, simple, safe, and efficient in vivo nucleic acid delivery system. The ideal system would include a nonviral carrier as well as a methodologic approach that would be specific for the liver, minimally invasive, and with the potential to be performed in an outpatient setting.

This video can be viewed directly from the GIE website or by using the QR code and your mobile device. Download a free QR code scanner by searching "QR Scanner" in your mobile device's app store.

The sleeping beauty (SB) transposon system has been used to promote the integration of transgenes in mammalian cells via a cut-and-paste mechanism.[5] The system has found its applications mainly in small animals with few in vivo large animal[6] or human studies.[7] The system consists of plasmids containing 2 transcription units, 1 expressing the enzyme SB transposase and the other expressing the transgene DNA to be inserted into the host genome. In rodents, this technique has resulted in successful expression of coagulation factor IX,[8] factor VIII,[9] α_1-antitrypsin,[10] and many other proteins such that short-term correction of the diseased phenotype was observed. A critical requirement of nonviral gene delivery vehicles, such as SB, is a method to introduce the plasmids in the nucleus of target cells. Several methods have been investigated, mostly in small animals, to deliver nonviral vectors to the liver.[11] The most promising, to date, appears to be hydrodynamic injection via a vein.[12-21] This vascular route, as documented,[22,23] is technically challenging, time-consuming, and by extension expensive and has, expectedly, cardiovascular side effects. These studies relied on creating relatively high hydrostatic pressure in the vascular bed that promoted plasmid uptake into the target cells.

Although intravascular hydrodynamic injection has been the most commonly used route,[12-16] the delivery of plasmids via the bile duct represents an alternative pathway but has only been evaluated in rodents, through invasive surgical approaches.[24-26] If intrabiliary delivery of plasmids via ERCP-guided hydrodynamic injection in a large animal model could overcome the current challenges faced by intravascular injection, this may promote the commencement of liver-targeted gene therapy in humans.

Herein, we describe, for the first time, an ERCP-directed hydrodynamic delivery of SB and associated plasmids to swine liver. The bile duct route has theoretical advantages over intravascular delivery including significantly smaller volume of injection, absence of adverse cardiorespiratory events, and reduced risk of systemic toxicity and of systemic dispersal of plasmids and, by extension, increased specificity. Additionally, there is some evidence that bile may contain fewer nucleases versus blood, and therefore the intrabiliary route offers the theoretical advantage of improved DNA stability.[27] In the current study, we selected pT3-EF1a-NICD, pT3-EF1a-AKT, and pT3-N90-beta-catenin to be delivered as target constructs because they have demonstrated the capability to be integrated into somatic cells in vivo with the guidance of pCMV-SB transposon.[28-30] The overarching goal of this study was to establish that ERCP-directed, hydrodynamically mediated, nonviral liver gene therapy is simple, effective, and safe and to provide the preclinical backdrop for further human clinical trials.

The specific objectives of this study were to ascertain the parameters necessary for intrabiliary-delivered hydrodynamic gene delivery, to demonstrate feasibility of liver cell transduction using ERCP, and to assess whether successful transduction results in stable expression of the delivered plasmid proteins. The long-term goal of our research is to establish a minimally invasive method of nonviral gene delivery to the liver.

METHODS

Animal and study conditions

Fifteen Yorkshire pigs (*Sus scrofa domestica*) weighing 40 to 50 kg and aged 4 to 6 months at study initiation were obtained from a commercial, closed-herd swine vendor (Archer Farms, Darlington, Md). Environmental acclimation was at 72°F ± 2°F, 30% to 70% relative humidity, and 14:10 hour light:dark cycle, and approximately 15 air changes per hour occurred for 1 week before study initiation. Swine were housed individually in 24-ft^2 indoor runs and fed Teklad Mini-swine diet (No. 8753; Harlan Tekland, Madison, Wisc). Water was provided ad libitum before study initiation. All experimental animal procedures were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University and were conducted in compliance with the Animal Welfare Act, applicable Animal Welfare Regulations, and the Guide for the Care and Use of Laboratory Animals at an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited facility.[31-33]

Determination of maximal tolerable injection parameters of the swine bile duct

Initially, we performed ex vivo experiments using standard endoscopic accessories on 3 swine livers to determine the maximal tolerated injection parameters necessary to rupture the biliary tree (for further details see Supplementary Fig. 1, available online at www.giejournal.org). These ex vivo studies were used to inform parameters for initial in vivo nonsurvival studies on 3 swine (Supplementary Fig. 2, available online at www.giejournal.org). After the swine were anesthetized, the duodenoscope (ED-3490 TK; Pentax, Montvale, NJ) was inserted through the mouth and positioned in the proximal duodenum in front of the biliary orifice. The common bile duct (CBD) was cannulated with an extraction balloon preloaded with a .035-inch hydrophilic guidewire (Dreamwire; Boston Scientific, Natick, MA). Selective biliary cannulation was technically simple and safe (no risk of pancreatitis) because the opening of the pancreatic duct is separate in swine. Under fluoroscopic guidance (Allura Xper FD20; Philips Medical Systems N.A., Bothell, Wash), 3-mL boluses of one third strength iohexol contrast medium (Omnipaque, 350 mg/mL; GE Health Co, Princeton, NJ) were injected into the biliary tree to opacify important landmarks (cystic duct, hepatic hilum, and main intrahepatic ducts). The guidewire was then inserted into the intrahepatic ducts, and an extraction balloon was advanced to the common hepatic duct