We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Liver Gene Therapy: Employing Surgery and Radiology for Translational Research

Luis Sendra, María José Herrero, Luis Martí-Bonmatí,
Eva M. Montalvá, Rafael López-Andújar,
Matteo Frasson, Eduardo García-Granero and
Salvador F. Aliño Pellicer

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.72665>

Abstract

Gene therapy is a therapeutic strategy that aims to employ nucleic acids as drugs for the transient or permanent treatment of inherited or acquired pathologies. Based on the type of vector employed for the gene transfer, gene therapy can be classified as viral gene therapy and nonviral gene therapy. Nonviral gene therapy is less efficient but safer than viral gene therapy. Hydrodynamic naked DNA transfer has shown great translational potential, achieving therapeutic levels of a human protein in the murine model. The translational process of the procedure has already been performed. Different radiologic and surgical approaches permitted pressurizing the liver *in vivo* by excluding its vascularization partially or totally. These approaches mediated a tissue rate of human alpha-1-antitrypsin protein translation (100–1000 copies per cell) close to those obtained with the mouse gold standard model in a safe mode that could be translated to human settings.

Keywords: gene therapy, liver, hydrodynamic, radiologic, catheterization, transplantation, surgery

1. Introduction

Gene therapy is a therapeutic strategy that uses nucleic acids, in any of their forms, as a drug for the transient or permanent treatment of inherited or acquired pathologies [1]. From a regulatory point of view, these drugs are considered in the European Regulation 1394/2007 and the Directive 2001/83/CE of the European Parliament. These, basically, establish the following:

“A gene therapy drug is a biologic drug with the following features:

- a. it includes an active principle that contains a recombinant nucleic acid, or it is constituted by this, and it is employed or administered in human beings, aiming to regulate, repair, substitute, add or remove a gene sequence;
- b. its therapeutic, prophylactic or diagnostic effect depends directly on the nucleic acid sequence or its expression product.”

Gene therapy consists of the transfer of a gene with clinical interest to the target tissue or organ of a patient. This transfer can be mediated by a vector or vehicle or employing naked DNA. Gene therapy aims that the gene reaches the target cells with sufficient molecular bioavailability for the host cell's decoding machinery to decode the gene sequence and produce the protein encoded by it [2].

The correct production of the protein encoded by the transferred gene would permit:

- a. adding a new function to the target cell
- b. recovering a lost or diminished function
- c. inhibiting or modulating an exacerbated function
- d. editing the genome to correct the production of a defective protein

1.1. Gene therapy strategies

There are different alternatives to perform the gene transfer. Depending on the resource employed for the delivery procedure, gene therapy can be classified as viral and nonviral.

1.1.1. Viral gene therapy

Viral gene therapy employs a viral vector to carry the DNA of interest to the nucleus of the target cell [3]. This viral vector consists of the sequence of a virus, integrative or not, without the pathogenic sequences (related with its replicative ability), which are substituted by the therapeutic gene of interest. This strategy takes advantage of the viral ability to access the cells and employ their decoding machinery to translate its own genome. Viral gene therapy offers the following advantages:

- a. It mediates a more efficient transfer of the gene;
- b. Its administration could be systemic since the virus structure protects the gene from the circulating nucleases;
- c. It permits developing permanent (employing viral vectors with the ability to integrate within the host cell genome) or transient (employing non-integrative viruses such as adenoviruses) therapies;
- d. It is possible to select the target cell since some viruses present tropism.

However, they also present disadvantages to be considered:

- a. Some viruses can induce intense immune responses, limiting the repeated doses (especially relevant in adenoviral vectors);

- b.** The exact place of the host genome where viruses integrate their genome is still unknown, hence being possible to alter the normal cell functions (insertional mutagenesis, tumor transformation);
- c.** Although not probable, it is possible that the viral particle without pathogenic features could recover them by genetic recombination, resulting in a potential risk for its clinical use.

1.1.2. Nonviral gene therapy

Nonviral gene therapy consists of the delivery of DNA mediated by the use of a nonviral vector [4–6] or the delivery of naked DNA [7], by physical procedures. With the aim of protecting the delivered DNA from its degradation exerted by circulating nucleases, different types of nonviral vectors have been designed. These vectors can facilitate the DNA (negative net charge) access into the cell through its plasmatic membrane, also negatively charged. Among the different models of nonviral vectors, we can find:

- a.** Liposomes—formed by the inclusion of DNA molecules within the lipid’s concentric layers. They have the ability to protect the gene and facilitate its cell internalization by endocytosis and/or fusion with the cell membrane in order to release the DNA inside the cell [6, 8].
- b.** Polyplexes [6, 9]—they employ biodegradable polymers that protect the DNA from the degradation mediated by DNAses. The use of cationic lipids or polymers permits the formation of complexes with the DNA (lipopolyplexes and polyplexes, respectively) that facilitates its cell internalization.

Disadvantages of nonviral vectors, they have limited utility because of their difficult formulation for clinical application. Their efficiency in ‘in vitro’ experiments is much higher than the efficiency observed in ‘in vivo.’ Furthermore, they have sometimes induced the immune response in patients.

Since viral gene therapy has offered good transfer efficiency but with the potential risk of immune reaction, or even that the recovery of virus infectivity and gene therapy mediated by nonviral vectors does not offer real advantages, physical procedures for efficient and safe naked DNA transfer have been developed. The most important alternatives of these strategies are:

- a.** Electroporation consists of augmenting the cell membranes’ permeability by employing electric pulses [6, 10, 11]. It increases the transfer efficiency but is more targeted to muscular tissue;
- b.** Sonoporation consists of the application of ultrasound on a biological tissue, mediating the formation of bubbles that create a stir able to destabilize transiently the cell membrane and facilitating the access of gene [6, 12];
- c.** Magnetofection based on the application of a magnetic field after the transfer of a gene linked to metallic particles in order to lead the product inside the tissue. This strategy has not demonstrated important improvement [13, 14];
- d.** Jet injection consists of the high-speed injection of particles. It is employed specifically in muscle tissue [15–17];
- e.** Hydrodynamic gene transfer is mediated by changes in cell permeability induced by the intravascular injection of DNA saline solution (hydrofection). This has proved to be one

of the most promising methods for naked DNA transfer and presents a great potential of clinical application in several different organs [18, 19].

1.2. Gene therapy in clinics

Since the approval of the first gene therapy clinical trial performed in patients in 1989, the number of these clinical trials, with little fluctuations, has increased constantly [20] achieving approximately 2600 in total (updated in November 2017) with a maximum rate of 169 clinical trials approved in 2015 (**Figure 1**).

The increasing use of gene therapy was possible, thanks to the development of gene constructs by employing different genes depending on the therapeutic application. Among the

Figure 1. Gene therapy clinical trials approved in the world. In this figure, the number of gene therapy clinical trials approved in the world each year since 1989 until August 2016 is shown. Source: www.wiley.co.uk/genmed/clinical [21].

most employed, those modulating the immune response (by activation or repression) stand out from the others. In clinical trials, the most employed strategy for gene transfer has been viral gene transfer (around 70%). Adenoviruses (A) due to their capacity to carry large genes and express them transiently and retroviruses (R) because of their ability to integrate the gene within the host genome permitting its long-term stable expression (suitable for inherited deficiencies) are the most employed. The most employed strategies of nonviral gene therapy in clinical trials have been the lipofection and naked DNA (N), since they are the safest (**Figure 2**).

However, when clinical trials employing naked DNA for monogenic inherited diseases are searched, only six trials are found and when considering genes encoding cytokines, only one is found. Despite the different strategies to vectorize the gene and the types of treatment employed, only 0.1% of all clinical trials employing gene therapy reached phase IV (**Table 1**).

Among all the gene therapy procedures, the one that achieved the most positive benefit-risk balance has been the naked DNA hydrodynamic transfer. Given the potential interest

Figure 2. Vectors used in gene therapy clinical trials. Source: www.wiley.co.uk/genmed/clinical [21].

Phase	Number of clinical trials	Ratio (%)
I	1409	57.2
I/II	500	20.3
II	429	17.4
II/III	24	1
III	91	3.8
IV	3	0.1
Single subject	5	0.2

Source: www.wiley.co.uk/genmed/clinical [21].

Table 1. Phases of gene therapy clinical trials.

of hydrodynamic gene therapy and the wide range of application in clinics (especially in the liver), the translational process of the technique has been performed from the successful murine model to human liver segments. The swine model permitted adapting the procedure for 'in vivo' liver transfer. Different radiologic and surgical approaches performed to improve the liver hydrodynamic gene transfer 'in vivo' will be discussed in this chapter.

2. Hydrodynamic naked DNA transfer

2.1. Hydrodynamic methodology

The possibility of expressing heterologous genes with high efficacy after the delivery of naked DNA was firstly described during the mid-1990s [22, 23]. In 1999, Zhang et al. [24] and Liu et al. [25] introduced the hydrodynamic gene transfer procedure. This procedure consisted of the rapid injection of a large volume (2 ml in 5–7 s) of saline solution bearing the gene of interest through the tail vein in the mouse (20 g average weight). The possibility of transferring naked DNA efficiently aroused a great interest among researchers and clinicians since the hydrodynamic procedure permitted expressing high levels of a heterologous protein, employing a safer strategy than viral gene therapy. Different research groups focused their efforts on improving the technique in order to be safer, more efficient and reproducible [26].

2.2. Hydrofection mechanism

In **Figure 3**, the sinusoid circulation within the liver before and after the retrograde injection of a saline solution containing a plasmid is shown. The gene solution injected through the tail vein reaches the liver in a retrograde sense and increases the pressure inside the

Figure 3. Schematic representation of sinusoid organization after retrograde hydrodynamic injection of gene solution. When a plasmid is injected, vessel pressure increases inducing the separation of endothelium cells. This permits the access of gene constructs to the Disse space and to the hepatocytes through the massive formation of endocytic vesicles.

vessel. This distends the wall mediating the transient separation of endothelium cells. When this occurs, the DNA leaves the blood vessels through the sinusoid pores and intercellular spaces and reaches the Disse space. From this space, the DNA can access the hepatocyte, the massive formation of endocytic vesicles playing a relevant role in this process. This DNA must reach the nucleus of the hepatocyte in order that the gene information delivered can be decoded. When this process takes place efficiently, the DNA is transcribed to RNA and this is translated to the protein, which is released into the bloodstream (in case of plasma proteins).

Employing this procedure, therapeutic plasma levels of alpha-1-protein were achieved for periods of more than 6 months in mice [27]. Nowadays, many gene therapy experiments for different pathology treatments are being studied in mice [28–30]. The possibility of achieving therapeutic levels of heterologous proteins after hydrodynamic human gene transfer in the murine model boosted the efforts of research teams to develop and adapt the procedures in larger animals aiming to translate it into the clinics, since the hemodynamic changes induced by the hydrodynamic injection are not compatible with its use in humans.

The perfusion conditions that permitted achieving the most efficient results in the mouse implied doubling the animal's volemia in a very short period of time. The procedure had to be necessarily adapted since larger animals would have not tolerated these conditions. The modifications of the procedure were directed to diminish the systemic hydrodynamic pressure. This was performed by transferring the gene of interest to the one and only target organ by image-guided catheterization procedures. Studies were performed in rats [31, 32] and rabbit [33] models but the results obtained were much less efficient than that observed in the mouse. The most recent efforts focused on developing models of liver hydrodynamic perfusion in pigs given their anatomical proximity with humans [34].

In the swine model, different strategies for minimally invasive gene transfer were designed through liver catheterization [35, 36]. Although these procedures proved to be safe, the efficiency achieved was not remarkable. Some authors highlighted the possibility that higher intravascular pressure within the liver could be required. For this reason, different strategies to block the venous backflow and employ more demanding perfusion conditions were studied. Levels of heterologous protein expression were not close to therapeutics in any case. After several works carried out by research groups around the world, no significant result was achieved [37–41]. However, given the huge interest of this procedure and its potential to be translated to human clinical practice, different groups evaluated minutely the molecular process of the transferred gene decoding in order to confirm or refuse this possibility. Evaluating at molecular level the detailed delivery, transcription and translation of a transferred gene permitted in identifying the step of the decoding process that limited the final efficacy in liver tissue and comparing this process in different animal models: mouse, pig and human. The best conditions of efficacy and safety for liver hydrodynamic gene therapy have been established in pig liver 'in vivo' (by catheterization and surgery) and the human liver 'ex vivo' (by catheterization in watertight segments). The methodology permitted comparing quantitatively the efficiency of different procedures of liver gene transfer. These procedures included partial and complete vascular exclusion aiming to pressurize the organ without affecting the systemic hemodynamics.

2.3. Therapeutic targets

Since gene transfer can deliver a gene functionally complete to the cell, it presents a great interest for the treatment of inherited metabolic diseases [42–45], such as alpha-1-antitrypsin deficiency [46], in which the entire functional gene could be implemented.

Gene therapy can also play an important role in the treatment of different acquired pathologies. Its application for modulating the immune response in different proinflammatory conditions, such as liver transplantation, has been studied by implementing genes of anti-inflammatory cytokines such as interleukin-10 (IL10).

3. Clinical translation of hydrodynamic gene therapy

Several animal models have been employed for hydrodynamic gene transfer. The murine model has resulted in the gold standard of the procedure since therapeutic levels of the protein encoded by the transferred gene have been achieved. The translational process has been carried out in rat, rabbit, guinea pig, dog, pig and human liver segments.

The murine model consisted of the rapid injection of gene saline solution in a volume equivalent to the animal volemia. This large volume facilitates the backflow of the gene solution and provokes its retrograde access to the liver. The high heart rate of the mouse permits the injection of such volumes with animal survival. Similar conditions to those employed for mice were carried out in rats, although different adaptations for diminishing the solution volume have been proposed in order to follow up the translation process. Other researchers [47] studied different strategies to improve hydrodynamic gene delivery efficiency by targeting the right lateral liver lobe of the rat through the portal vein branch. The need for outflow blockade in the target area was reported since the portal vein pressure was too low to avoid backflow. In another attempt to improve the efficiency of the procedure, the left liver lobe was targeted in the rat and outflow occlusion was performed to compare its effect to free-flow control rats [32]. It was reported that outflow blockade is demanding to obtain efficient outcomes in transgene expression. Larger animals do not have the ability to increase the heart rate as mice and doubling their volemia would be incompatible with survival. Thus, the hydrodynamic injection had to be adapted to reduce the final volume and minimize the systemic hemodynamic impact. These adaptations focused on targeting an organ. Regarding this fact, Eastman et al. injected a gene to a single liver lobe employing a balloon catheter and to the entire organ of the rabbit with hepatic venous occlusion and achieved protein plasma expression in 2 days. The safety of the liver hydrodynamic gene transfer was also assessed in dogs to prove its feasible application in large animals [48]. They performed four successive injections in four different main liver lobes. Authors observed no significant harmful effects and rapid recovery of animals. However, the results obtained were poor.

The following step for the clinical translation of the procedure was to test its potential use in anatomically more similar animals to human beings such as pigs and primates. The techniques for gene delivery that were employed should be applicable in human settings.

Yoshino et al. [35] and Aliño et al. [36] described the first attempts performed in a pig. The total volume employed was reduced by targeting an area of liver and compared different

catheter-mediated delivery strategies. These strategies included portal vein occlusion, left hepatic artery occlusion, portal vein and left hepatic artery occlusion and both vessels' occlusion with blood flow washout. Yoshino et al. injected the gene solution through the cava vein. The occlusion of portal vein and hepatic artery with the washout mediated the most efficient outcomes achieving disperse protein plasma levels for several weeks. For the first time, the procedure showed interesting results in pigs, for those proteins with low expression. In another work, hydrodynamic retrovenous gene transfer was performed in large and small areas of pigs' liver. Alino et al. [36] reported the presence of gene and protein expression in tissues, mainly within the perivenous area. Targeting smaller areas but employing same volumes of gene solution, higher plasma protein levels were achieved, much lower than those considered therapeutic. Fabre et al. [37] targeted the entire liver and isolated the hepatic segment of the inferior vena cava by clamping it suprahepatically and infrahepatically. Gene solution was transferred by a hydrodynamic procedure through two parallel syringes and, although the efficiency of gene delivery was much lower than the one observed in the mouse and rat, they confirmed the clinical feasibility of the technique as determined by systemic blood pressures, ECG, heart rate and so on.

Pressure reached within the liver during the hydrodynamic injection played an important role. For this reason, Fabre et al. [40] focused their work on pressurizing individual lobes of the liver by isolating them. Aiming to achieve localized high pressure without affecting the systemic circulation, they proposed individualizing the lobe by employing catheters with balloon and ligation. Although most of the authors suggested blood pressure to be the most important feature of hydrodynamic injection for efficient gene transfer, others have pointed other characteristics such as impulse [49] and flow rate [50, 51] to be relevant. However, nearly all authors agree to the need for isolating target areas or the entire liver to improve the procedure efficiency. This vascular isolation could be partial or complete.

Firstly, the implication of the complete vascular exclusion in the final efficacy of the procedure should be evaluated in order to determine its relevance. As previously reported, the complete liver vascularization of the pig can be occluded up to 20 min without neither hepatic injury nor systemic damage [52]. Considering this fact, Carreño et al. [50] described in pigs a surgical procedure to completely exclude liver vascularization 'in vivo' and perform hydrodynamic gene delivery, targeting the entire organ. A complete midline laparotomy was carried out, exposing all the abdominal organs. The clamping sequence was as follows: first, the hepatic artery, then the portal vein and finally the infrahepatic vena cava, to interrupt hepatic inflow. The suprahepatic vena cava was clamped last, to secure total hepatic vascular exclusion. Depending on the flow sense of gene transfer, three different models were designed. In model 1 the portal vein was clamped, and only a longitudinal incision was made on the anterior surface of the cava vein to insert the perfusion cannula. In model 2, the process was the same as in model 1 but with the clamping of the vena cava and perfusion through the portal vein. In model 3 (**Figure 4**), the gene solution was injected simultaneously through suprahepatic IVC (Inferior Vena Cava) and the portal vein employing two catheters connected by a Y connector and a high-volume pump. After solution perfusion, the liver was kept under total vascular exclusion for no more than 5 min to allow gene penetration into the cell nuclei.

In all three models, when suprahepatic IVC was occluded and liver vasculature was completely excluded, the systemic pressure decreased rapidly. However, 1 min after revascularization this parameter was entirely normalized and animals recovered in few hours. Due to the invasiveness of the surgical procedure that included a laparotomy, same authors designed

a technique for liver venous sealing mediated by image-guided catheterization [53]. Two strategies with different degrees of liver vasculature closure were proposed:

- a. Inject the gene solution through a balloon catheter placed in a single lobe (**Figure 5**), and only target this part of the liver and
- b. Place simultaneously three catheters with balloons within suprahepatic IVC, infrahepatic IVC and portal vein around the liver entry (**Figure 6**) in order to close its vasculature. The gene solution is injected through the catheter placed in suprahepatic IVC and the entire organ is targeted.

These three procedures, surgery and open and closed catheterization, proved to be safe. After gene transfer and animal awakening, their recovery was very fast and presented normal

Figure 4. Schematic figure of liver simultaneous cava and porta perfusion with laparotomy surgery. Suprahepatic inferior vena cava and hepatic artery are ligated, and gene solution is transferred simultaneously by portal vein and infrahepatic inferior vena cava. Modified from [50].

Figure 5. Single-lobe catheterization by balloon-catheter. Left panel is a schematic figure of catheter localization. Only the hepatic vein employed for gene transfer is occluded. Suprahepatic IVC, infrahepatic IVC and portal vein are not closed. Right panel shows two radiologic images of catheter position and iodinated contrast solution injection in single lobes defining the area affected by solution injected. The gene solution is injected through hepatic veins. The backflow is blocked by inflated balloon [54].

Figure 6. Whole liver catheterization by balloon-catheters. Upper panel is a schematic figure of catheters localization. Infrahepatic IVC and portal vein are closed at liver access by inflated balloon-catheters. Lower left panel shows a radiologic image of supra and infrahepatic IVC catheters position. Lower mid panel shows an inflated balloon-catheter placed at portal vein blocking its exit. Lower right panel shows the iodinated contrast solution injection in the entire liver. The gene solution is injected through suprahepatic inferior vena cava [54].

behavior few hours after the intervention. Furthermore, all of them mediated tissue expression of the protein encoded by the transferred gene. The rate of protein translation showed a direct relation with the degree of vasculature closure: surgery-mediated complete liver vasculature exclusion > catheterization-mediated venous vasculature closure > catheterization-mediated single lobe without organ vasculature closure.

Transferring the human alpha-1-antitrypsin, the single liver lobe strategy mediated 20,000 copies of protein per cell in the liver. Targeting the entire organ with the closure of suprahepatic IVC, infrahepatic IVC and portal vein mediated a higher translation rate up to 100,000 copies per cell. The complete exclusion of liver vasculature by occlusion IVC, portal vein and hepatic artery with surgical procedure increased this rate up to 400,000 copies per cell in the liver tissue. The highest rate of tissue translation achieved was only 10-fold lower than the one obtained with the successful gold standard procedure performed in the mouse. This suggests that the hydrodynamic procedure of liver gene therapy with vascular exclusion mediated by radiological and surgical strategies mediated efficient delivery with efficacious translation protein.

Once proved the efficiency of these procedures in pig and the confirmation of their safety for gene transfer 'in vivo,' the following step of translational process consisted of demonstrating the efficacy in human liver tissue.

In this sense, human liver segments proceeding from surgical resection in patients with cancer were injected with different genes to evaluate the potential transferability of this technique. Given their precedence, the vasculature of these human liver segments is entirely excluded so they are watertight and hence, pressurized. The gene is retrogradely transfected through a catheter placed in a hepatic vein (**Figure 7**) and the segment remains watertight for 5 min.

The first studies of gene transfer with human liver segments [56] used the eGFP tracer gene in order to easily determine its expression efficacy, and it was demonstrated that the gene could be efficiently delivered and the protein was produced within the liver tissue as observed by fluorescence microscopy. After confirming the feasibility of the technique in this type of tissue, genes with clinical interest were employed to define the translational potential to clinical real settings.

Sendra Gisbert et al. [55] transferred a plasmid bearing the human interleukin-10 gene (IL10). Interleukin-10 is an immunomodulatory protein with pleotropic effects with potential interest for the treatment of inflammatory diseases or for inducing tolerance in organ transplantation. The rate of tissue protein translation achieved was around 1000 copies per cell, this meaning the potential therapeutic production of protein (IC₅₀ of IL10 for TNF α = 124 pg) if compared with other results of the same group.

Our group also transferred in similar human liver segments a plasmid with the same human alpha-1-antitrypsin employed in mice and pigs but modified. In order to permit differing endogenous and exogenous genes and proteins, a sequence of nucleotides encoding the flag peptide was added. Preliminary experiments demonstrate that the procedure is efficient and the use of a human gene in human tissue favors the production of protein. First, results prove a rate of tissue protein translation of 10^4 – 10^5 copies of hAAT-flag protein per cell, this accounting for up to 22% of all the hAAT proteins present in the liver tissue in 1 week.

The efficacy of gene transfer can be measured by different techniques and authors have studied many variables to present their results and evaluate how efficient a procedure is. This requires the use of a more detailed analysis that allows to identify the effectiveness of each of the stages of the process of delivery of the gene, its decoding of protein and its subsequent location.

Figure 7. Catheterization of human liver segment. Left panel shows a schematic figure of a human liver segment with a catheter placed in a hepatic vein. Right panel is a radiographic image of a human liver segment injected with iodinated contrast solution through hepatic vein. Modified from [55].

The molecular quantitative evaluation of decoding is demanding for a correct interpretation of the process. Quantitative determination of the molecular process provides real data of delivery, transcription and translation indexes. It would be important that researches achieved an agreement in data quantitation and expression to be able to objectively compare results and define the better conditions for gene transfer. The units should be expressed in molecular units (as number of copies or moles) or other units of mass. It is also very important that the data are referred to a common circumstance, such as a standard or 'normalized cell.' The normalized cell is defined as 'typical mammalian hepatocyte with defined content of total DNA' (genome weight of each specific animal, for instance, human: 6.6 pg), RNA (20 pg) and protein (500 pg).

This strategy offers an objective analysis that permits expressing the data as the copy number of each molecular specie, considering the standard content of DNA, RNA and protein in a normalized cell. This offers a more comprehensive interpretation of the entire process and permits comparing the results among different works and research groups.

To sum up, the hydrodynamic procedure is an efficient strategy for gene delivery demonstrated by the levels of tissue protein that is observed. The more the vasculature is occluded, the better is the final protein expression. The surgical procedure permits, excluding liver, entire vasculature and mediates the higher expression rate. However, non-invasive image-guided catheterization permits good levels of protein production without the need of a laparotomy incision.

Acknowledgements

This work has been partially supported by Spanish Ministerio de Economía y Competitividad (SAF2011-27002, SAF2007-64492) and Grifols ALTA Award 2017.

Author details

Luis Sendra^{1,2}, María José Herrero^{1,2*}, Luis Martí-Bonmatí³, Eva M. Montalvá⁴, Rafael López-Andújar⁴, Matteo Frasson⁵, Eduardo García-Granero⁵ and Salvador F. Aliño Pellicer^{1,2}

*Address all correspondence to: maria.jose.herrero@uv.es

1 Pharmacology Department, Medicine Faculty, University of Valencia, Valencia, Spain

2 Pharmacogenetics Unit, La Fe Health Research Institute, La Fe University and Polytechnic Hospital, Valencia, Spain

3 Radiology Department and Biomedical Imaging Research Group (GIBI230), La Fe University and Polytechnic Hospital, Valencia, Spain

4 Unit of Hepato-Biliary-Pancreatic Surgery and Transplantation, La Fe University and Polytechnic Hospital, Valencia, Spain

5 Department of General Surgery, Digestive Surgery Unit, La Fe University and Polytechnic Hospital, Valencia, Spain

References

- [1] Kresina TF, Branch AD. In: Kresina TF, editor. *An Introduction to Molecular Medicine and Gene Therapy*. New York: Wiley-Liss; 2001. pp. 1-24
- [2] Kay MA. State-of-the-art gene-based therapies: The road ahead. *Nature Reviews Genetics*. 2011;**12**:316-328
- [3] Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: The art of turning infectious agents into vehicles of therapeutics. *Nature Medicine*. 2001;**7**(1):33-40
- [4] Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Robert Dorkin J, Anderson DG. Non-viral vectors for gene-based therapy. *Nature Reviews Genetics*. 2014;**15**(8):541-555
- [5] Ibraheem D, Elaissari A, Fessi H. Gene therapy and DNA delivery systems. *International Journal of Pharmaceutics*. 2014;**459**(1-2):70-83
- [6] Kamimura K, Suda T, Zhang G, Liu D. Advances in gene delivery systems. *Pharmaceutical Medicine*. 2011;**25**(5):293-306
- [7] Taniyama Y, Azuma J, Kunugiza Y, Iekushi K, Rakugi H, Morishita R. Therapeutic option of plasmid-DNA based gene transfer. *Current Topics in Medicinal Chemistry*. 2012;**12**(15):1630-1637
- [8] Dasí F, Benet M, Crespo J, Crespo A, Aliño SF. Asialofetuin liposome-mediated human alpha 1-antitrypsin gene transfer in vivo results in stable long-term gene expression. *Journal of Molecular Medicine*. 2001;**79**:205-212
- [9] Chemin I, Moradpour D, Wieland S, et al. Liver-directed gene transfer: A linear polyethylenimine derivative mediates highly efficient DNA delivery to primary hepatocytes in vitro and in vivo. *Journal of Viral Hepatitis*. 1998;**5**:369-375
- [10] Li S. Electroporation gene therapy. Preface. *Methods in Molecular Biology*. 2008;**423**:v-vii
- [11] Heller LC, Heller R. Electroporation gene therapy preclinical and clinical trials for melanoma. *Current Gene Therapy*. 2010;**10**(4):312-317
- [12] Tomizawa M, Shinozaki F, Motoyoshi Y, Sugiyama T, Yamamoto S, Sueishi M. Sonoporation: Gene transfer using ultrasound. *World Journal of Methodology*. 2013;**3**(4):39-44
- [13] Plank C, Anton M, Rudolph C, et al. Enhancing and targeting nucleic acid delivery by magnetic force. *Expert Opinion on Biological Therapy*. 2003;**3**:745-758
- [14] Dobson J. Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. *Gene Therapy*. 2006;**13**:283-287
- [15] Yang NS, Burkholder J, Roberts B, et al. In vivo and in vitro gene transfer to mammalian somatic cells by particle bombardment. *Proceedings of the National Academy of Sciences of the United States of America*. 1990;**87**:9568-9572
- [16] Wang S, Joshi S, Lu S. Delivery of DNA to skin by particle bombardment. *Methods in Molecular Biology*. 2004;**245**:185-196

- [17] Langer B, Renner M, Scherer J, Schüle S, Cichutek K. Safety assessment of biolistic DNA vaccination. *Methods in Molecular Biology*. 2013;**940**:371-388
- [18] Herweijer H, Wolff JA. Gene therapy progress and prospects: Hydrodynamic gene delivery. *Gene Therapy*. 2007;**14**(2):99-107
- [19] Suda T, Liu D. Hydrodynamic gene delivery: Its principles and applications. *Molecular Therapy*. 2007;**15**:2063-2069
- [20] Ginn SL, Alexander IE, Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2012—An update. *The Journal of Gene Medicine*. 2013;**15**:65-77
- [21] Wiley database on Gene Therapy Trials Worldwide. <http://www.wiley.com/legacy/wileychi/genmed/clinical/>
- [22] Budker V, Zhang G, Knechtle S, Wolff JA. Naked DNA delivered intraportally expresses efficiently in hepatocytes. *Gene Therapy*. 1996;**3**:593-598
- [23] Zhang G, Vargo D, Budker V, Armstrong N, Knechtle S, Wolff JA. Expression of naked plasmid DNA injected into the afferent and efferent vessels of rodent and dog livers. *Human Gene Therapy*. 1997;**8**:1763-1772
- [24] Zhang G, Budker V, Wolff JA. High levels of foreign gene expression in hepatocytes after tail vein injections of naked plasmid DNA. *Human Gene Therapy*. 1999;**10**:1735-1737
- [25] Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Therapy*. 1999;**6**:1258-1266
- [26] Wang Z, Qiu SJ, Ye SL, Tang ZY, Xiao X. Combined IL-12 and GM-CSF gene therapy for murine hepatocellular carcinoma. *Cancer Gene Therapy*. 2001;**8**(10):751-758
- [27] Aliño SF, Crespo A, Dasí F. Long-term therapeutic levels of human alpha-1 anti-trypsin in plasma after hydrodynamic injection of nonviral DNA. *Gene Therapy*. 2003;**10**(19):1672-1679
- [28] Gao M, Ma Y, Cui R, Liu D. Hydrodynamic delivery of FGF21 gene alleviates obesity and fatty liver in mice fed a high-fat diet. *Journal of Controlled Release*. 2014;**185**:1-11
- [29] Ma Y, Liu D. Hydrodynamic delivery of adiponectin and adiponectin receptor 2 gene blocks high-fat diet-induced obesity and insulin resistance. *Gene Therapy*. 2013;**20**(8):846-852
- [30] Sun H, Liu D. Hydrodynamic delivery of interleukin 15 gene promotes resistance to high fat diet-induced obesity, fatty liver and improves glucose homeostasis. *Gene Therapy*. 2015;**22**(4):341-347
- [31] Zhang X, Collins L, Sawyer GJ, Dong X, Qiu Y, Fabre JW. In vivo gene delivery via portal vein and bile duct to individual lobes of the rat liver using a polylysine-based nonviral DNA vector in combination with chloroquine. *Human Gene Therapy*. 2001;**12**:2179-2190
- [32] Sawyer GJ, Zhang X, Fabre JW. Technical requirements for effective regional hydrodynamic gene delivery to the left lateral lobe of the rat liver. *Gene Therapy*. 2010;**17**:560-564

- [33] Eastman SJ, Baskin KM, Hodges BL, et al. Development of catheter-based procedures for transducing the isolated rabbit liver with plasmid DNA. *Human Gene Therapy*. 2002;**13**:2065-2077
- [34] Herrero MJ, Dasi F, Noguera I, et al. Mouse and pig nonviral liver gene therapy: Success and trials. *Gene Therapy and Molecular Biology*. 2005;**9**:169-180
- [35] Yoshino H, Hashizume K, Kobayashi E. Naked plasmid DNA transfer to the porcine liver using rapid injection with large volume. *Gene Therapy*. 2006;**13**:1696-1702
- [36] Aliño SF, Herrero MJ, Noguera I, Dasi F, Sánchez M. Pig liver gene therapy by noninvasive interventionist catheterism. *Gene Therapy*. 2007;**14**:334-343
- [37] Fabre JW, Grehan A, Whiterhorne M, et al. Hydrodynamic gene delivery to the pig liver via an isolated segment of the inferior vena cava. *Gene Therapy*. 2008;**15**:452-462
- [38] Sawyer GJ, Rela M, Davenport M, Whitehorne M, Zhang X, Fabre JW. Hydrodynamic gene delivery to the liver: Theoretical and practical issues for clinical application. *Current Gene Therapy*. 2009;**9**(2):128-135
- [39] Kamimura K, Suda T, Xu W, Zhang G, Liu D. Image-guided, lobe-specific hydrodynamic gene delivery to swine liver. *Molecular Therapy*. 2009;**17**(3):491-499
- [40] Fabre JW, Whitehorne M, Grehan A, et al. Critical, physiological and surgical considerations for hydrodynamic pressurization of individual segments of the pig liver. *Human Gene Therapy*. 2011;**22**(7):879-887
- [41] Kamimura K, Suda T, Zhang G, Aoyagi Y, Liu D. Parameters affecting image-guided, hydrodynamic gene delivery to swine liver. *Molecular Therapy Nucleic Acids*. 2013;**2**:e128
- [42] Strauss M. Liver-directed gene therapy: Prospects and problems. *Gene Therapy*. 1994;**1**:156-164
- [43] Davern TJ. Molecular therapeutics of liver disease. *Liver Disease*. 2001;**52**:381-414
- [44] O'Connor TP, Crystal RG. Genetic medicines: Treatment strategies for hereditary disorders. *Nature Reviews Genetics*. 2006;**4**:261-282
- [45] Brunetti-Pierri N, Ng P. Progress & prospects: Gene therapy for genetic diseases with helper-dependent adenoviral vectors. *Gene Therapy*. 2008;**15**:553-560
- [46] Janciauskiene SM, Bals R, Koczulla R, Vogelmeier C, Köhnlein T, Welte T. The discovery of α 1-antitrypsin and its role in health and disease. *Respiratory Medicine*. 2011;**105**(8):1129-1139
- [47] Zhang X, Dong X, Sawyer GJ, Collins L, Fabre JW, Zhang X, et al. Regional hydrodynamic gene delivery to the rat liver with physiological volumes of DNA solution. *The Journal of Gene Medicine*. 2004;**6**(6):693-703
- [48] Kamimura K, Kanefuji T, Yokoo T, Abe H, Suda T, Kobayashi Y, Zhang G, Aoyagi Y, Liu D. Safety assessment of liver-targeted hydrodynamic gene delivery in dogs. *PLoS One*. 2014;**9**(9):e107203

- [49] Hackett PB Jr, Aronovich EL, Hunter D, Urness M, Bell JB, Kass SJ, Cooper LJ, McIvor S. Efficacy and safety of sleeping beauty transposon-mediated gene transfer in preclinical animal studies. *Current Gene Therapy*. 2011;**11**(5):341-349
- [50] Carreño O, Sendra L, Montalvá E, Miguel A, Orbis F, Herrero MJ, Noguera I, Aliño SF, Lopez-Andujar R. A surgical model for isolating the pig liver in vivo for gene therapy. *European Surgical Research*. 2013;**51**(1-2):47-57
- [51] Sendra L, Carreño O, Miguel A, Montalvá E, Herrero MJ, Orbis F, Noguera I, Barettino D, López-Andújar R, Aliño SF. Low RNA translation activity limits the efficacy of hydrodynamic gene transfer to pig liver in vivo. *The Journal of Gene Medicine*. 2014;**16**(7-8):179-192
- [52] de Groot GH, Reuvers CB, Schalm SW, Boks AL, Terpstra OT, Jeekel H, ten Kate FW, Bruinvels J. A reproducible model of acute hepatic failure by transient ischemia in the pig. *The Journal of Surgical Research*. 1987;**42**(1):92-100
- [53] Sendra L, Miguel A, Pérez-Enguix D, Herrero MJ, Montalvá E, García-Gimeno MA, Noguera I, Díaz A, Pérez J, Sanz P, López-Andújar R, Martí-Bonmatí L, Aliño SF. Studying closed hydrodynamic models of “In Vivo” DNA perfusion in pig liver for gene therapy translation to humans. *PLoS One*. 2016;**11**(10):e0163898
- [54] Sendra L, Pérez D, Miguel A, Herrero MJ, Noguera I, Díaz A, Barettino D, Martí-Bonmatí L, Aliño SF, Human AAT. Gene transfer to pig liver improved by using a perfusion isolated organ endovascular procedure. *European Radiology*. 2016;**26**(1):95-102
- [55] Sendra Gisbert L, Miguel A, Sabater L, Herrero MJ, Sabater L, Montalvá EM, Frasson M, Abargues R, López-Andújar R, García-Granero E, Aliño SF. Efficacy of hydrodynamic interleukin 10 gene transfer in human liver segments with interest in transplantation. *Liver Transplantation*. 2017;**23**(1):50-62
- [56] Herrero MJ, Sabater L, Guenechea G, Sendra L, Montilla AI, Abargues R, Navarro V, Aliño SF. DNA delivery to ‘ex vivo’ human liver segments. *Gene Therapy*. 2012;**19**(5):504-512