Gene transfer as treatment for metabolic inherited liver diseases

José Luiz de Godoy*

Abstract

Objective: to study gene transfer aiming at its future clinical applications in the treatment of inborn metabolic diseases of the liver.

Methods: review of the literature.

Results and Conclusions: gene transfer into the liver can be an alternative to liver transplantation in the treatment of inborn metabolic diseases. Various vectors have been described in gene transfer, including retrovirus vectors, whose integration into the chromosomal DNA can allow stable, long-term expression of the transgene. The integration of retrovirus vectors into the genome of the target cell is possible only during mitosis. Therefore, these vectors must be delivered during hepatic regeneration induced by partial heptectomy, for example. Another obstacle that needs to be overcome is avoiding extrahepatic dissemination of retroviruses, especially to germinal cells, since that would present the risk of changing the genetic heritage of descendants.


Introduction

The identification and cloning of genes directly related to, or involved in onset of inborn or acquired diseases have given rise to new therapeutic perspectives with the use of intracellular gene transfer.1

The objective of gene transfer is to introduce a foreign gene whose expression will: (i) restore the genetically defective function or allow cells to acquire a new metabolic function by transfer of a normal gene into somatic cells affected by gene mutation or by a disturbance of gene transcription or transduction; and (ii) accomplish, through a correspondingly secreted protein, death of the target cancer cell by delivering a suicide gene whose expression leads to cell death by production of pro-drugs.2,3

Three advantages of liver-directed gene transfer are: first, that the liver is the site of numerous different protein syntheses and, consequently, of enzyme anomalies or deficiencies potentially curable by gene transfer; second, liver cell culture allows in vitro studies of gene transfer into hepatocytes; and third, hepatic surgery allows, with minimal risk, the design of surgical procedures that would facilitate liver-directed gene transfer.4 Gene transfer should be proven effective and innocuous in experimental models before it is used in humans.

* Docteur ès Sciences, Université Paris V (René Descartes), Paris, France. Pediatric Surgeon, Hospital de Clínicas, Universidade Federal do Paraná (UFPR), Curitiba, PR.
Inborn metabolic diseases of the liver

Currently, liver transplantation is indicated for a significant number of inborn metabolic diseases of the liver (Table 1). It allows the normal enzymatic activity to restore and also effectively cures the metabolic disease.5,6

Metabolic diseases caused by direct lesion of the liver

Among metabolic diseases, there is a group in which the enzymatic abnormality causes early and serious hepatopathies, which evolve to cirrhosis or to risk for liver cancer. Presently, during the cirrhosis stage of metabolic diseases, liver transplantation seems to be the only viable solution. Liver transplantation allows both the restoration of enzyme activity and the replacement of the liver, whose lesion is the main aspect of patient symptomatology.7 It seems that gene transfer alone could not produce a significant therapeutic effect on lesions caused by cirrhosis. According to Overturf et al.,8 only early diagnosis of the disease would allow efficient treatment with gene transfer. The authors carried out a study with a mouse model of hereditary tyrosinemia type I (HT1) whose gene coding for fumarylacetoacetate hydrolase (FAH) was invalidated. This disease affects infants by subsequent acute hepatocellular failure, associated with high morbidity rates during the first months of life, or by cirrhosis, frequently associated with rickets during the second semester of life. The disease also presents a high risk for hepatocellular carcinoma among infants. Overturf et al. demonstrated the advantage of \textit{in vivo} selection of hepatocytes expressing fumarylacetoacetate hydrolase enzyme (normal hepatocytes) in relation to hepatocytes that do not present this enzyme (mutant hepatocytes) for the correction of the phenotype. The absence of normal enzymatic activity causes accumulation of metabolites that are toxic for the hepatocytes, resulting in a predisposition to permanent hepatocyte regeneration. In this process, normal hepatocytes are positively selected in relation to mutant hepatocytes. After transplantation into the spleen of the mouse model, normal hepatocytes were able to repopulate over 80% of the mutant liver. The minimum number of transplanted wild-type hepatocytes necessary for repopulating 50% of the mutant liver was 1,000 hepatocytes. In this same study, mutant hepatocytes corrected \textit{in situ} by retroviral gene transfer with FAH were also positively selected in relation to non-transduced mutant hepatocytes. Two months after retrovirus infusion into the portal vein, over 90% of hepatocytes became FAH positive and liver function was restored to normal8.

Table 1 - Indication of liver transplantation for inborn metabolic diseases of the liver

<table>
<thead>
<tr>
<th>Disease</th>
<th>Deficiency of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1 antitrypsin deficit</td>
<td>alpha-1 antitrypsin</td>
</tr>
<tr>
<td>Hereditary tyrosinemia</td>
<td>fumarylacetoacetate hydrolase</td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td>ceruloplasmin</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>galactose-1-phosphate-uridylytransferase</td>
</tr>
<tr>
<td>Type 1 glycogenosis</td>
<td>glucose 6-phosphatase</td>
</tr>
<tr>
<td>Type 4 glycogenosis</td>
<td>alpha-1, 4-glucan 6-glucosyltransferase</td>
</tr>
<tr>
<td>Protoporphyria</td>
<td>porphobilinogen deaminase</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>CFTR</td>
</tr>
</tbody>
</table>

Metabolic diseases with serious extrahepatic complications

<table>
<thead>
<tr>
<th>Disease</th>
<th>Deficiency of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxaluria type 1</td>
<td>peroxisomal alanine-glyoxylate-aminotransferase</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>low density lipoprotein receptors (LDLRs)</td>
</tr>
<tr>
<td>Crigler-Najjar type 1</td>
<td>bilirubin uridine diphosphate-glucuronosyltransferase</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>factor VIII</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>factor IX</td>
</tr>
<tr>
<td>Protein C deficit</td>
<td>protein C</td>
</tr>
<tr>
<td>Urea-cycle</td>
<td>ornithine-transcarbamylase</td>
</tr>
<tr>
<td></td>
<td>argininosuccinate synthetase</td>
</tr>
</tbody>
</table>

Adapted from Bernard et al.,7 Esquivel et al.,5 Panis et al.,4 and Sherlock & Dooley.6
In this model, *in vivo* selection was based on: (i) the competitive growth advantage of normal hepatocytes in relation to mutant hepatocytes with deficiency of FAH; (ii) permanent hepatic regeneration related to accumulation of metabolites that are toxic for mutant hepatocytes; (iii) the fact that these metabolites are not toxic for normal hepatocytes.

In a more recent study, the same authors showed that the isolated mutant hepatocytes of the mouse’s FAH-deficient liver could be transduced into cellular culture by retroviral vectors presenting this enzyme’s gene. After re-implantation into the spleen, the transduced hepatocytes were positively selected in relation to mutant hepatocytes, resulting in a repopulation of over 85% of the liver.

The principle of liver repopulation with positive selection is applicable to other animal models of metabolic diseases and, in theory, to humans, for the treatment of lethal metabolic diseases resulting from direct lesion of the liver.

**Metabolic diseases with extra-hepatic complications**

A second group of diseases is characterized by presenting serious extra-hepatic outcomes. These diseases can present poor prognosis, but with no related hepatic symptoms (Table 1). In this second group of metabolic diseases, which are caused by a monogenic mutation and have a well-known molecular base, the correction (even partial) of enzymatic mutation would improve the phenotype of the disease. In this sense, liver-directed gene transfer could represent a therapeutic alternative to liver transplantation in the treatment of the clinical outcome of these metabolic diseases.

Actually, in most part of enzymatic abnormalities, manifestation of clinical outcomes is caused by reduced enzymatic activity, since 5 to 25% of normal enzymatic activity is usually enough to protect against the clinical manifestations. In this sense, hemophilia B could be taken as an example, since this disease is caused by mutation of the coagulation factor IX (F.IX). Individuals with less than 1% of normal F.IX activity have a significant risk for spontaneous hemorrhage; those with 1 to 10% of normal F.IX activity have a moderate risk for spontaneous hemorrhage; and those with 10 to 20% of normal F.IX activity may not present any clinical manifestation of the disease.

**Gene transfer strategies**

Two procedures may be considered in theory for correction of gene mutation by transfer of a gene into somatic cells: gene replacement (*in situ* correction) and gene increase (ectopic transplantation).

**Gene replacement**

Gene replacement consists of correction of the genotype through the correction of a specific gene abnormality. In gene replacement, the abnormal gene is selectively removed and replaced by a normal gene. This would be a procedure of choice since the intervention is *in situ*, with the corrected gene remaining in its natural genomic environment and submitted to normal regulatory mechanisms. Currently, however, this procedure is still unfeasible, since the means for correcting a specific lesion in a specific gene are not well-determined yet.

**Ectopic transplantation**

The objective of ectopic transplantation is the correction of the phenotype by inserting a normal gene into a random site of the genome. This procedure consists of adding a functional gene without moving the abnormal gene. The transduced gene may not be accepted and remain in episomal form. At the present moment, ectopic transplantation is the only procedure that proved effective and relatively innocuous in animal models. The use of ectopic transplantation has been allowed in humans in protocols of gene therapy.

**Germinative gene transfer or somatic gene transfer**

**Germinative gene transfer:** if gene correction is made on a germinal cell or in a young embryo cell, it will be transmitted to descendants. However, germinative gene transfer cannot be applied to human beings due to technical and, most importantly, ethical reasons, since it would result in an alteration of the genetic constitution of humans. On the other hand, it is a useful experimental model.

Germinal gene transfer can be used to create genetically modified animals, which express a gene that is usually not found in the animal studied.

**Somatic gene transfer:** if gene correction is made on somatic cells, it will cause a phenotypic correction to a specific group of cells without affecting the patient’s genetic constitution. Somatic gene transfer procedures in humans, a priori, raise the same ethical problems than organ or tissue transplant procedures do.

**Gene transfer elements**

There are three fundamental elements in gene transfer, namely the gene, the vector, and the target cell.

**The transferred gene**

The transferred gene must be understood, sequenced, and cloned before it can be used. It is used in the form of viral construction containing the transferred gene and/or an auxiliary gene (or selection gene), which brings a selective advantage. Transfer genes can be markers or have therapeutic importance.
Markers are naturally absent in the target cell. These genes allow the activation of the feasibility and of the status of gene transfer, as well as the assessment of the duration and level of transgene expression in the target cell. For example, the Escherichia coli beta-galactosidase gene (lac Z), together or not with a nuclear localization signal (nls lac Z), is widely used in viral construction. Its activity is identifiable by a histochemical reaction with X-Gal substratum, which confers an intense blue color to cells that express the transgene.\(^1\)\(^5\) The gene coding for GFP (green fluorescent protein) is also widely used. The spontaneous fluorescence of its expressed product can be detected, measured, and tracked in live cells without adding substratum.

Therapeutic genes, which are evidently used with therapeutic objectives, are those coding for proteins, hormones, or enzymes whose abnormal or nonexistent syntheses can be responsible for a well-known disease (for example, the mutation of the gene coding for F.IX of coagulation is responsible for hemophilia B).\(^1\)\(^6\)

**The vector**

Gene transfer is carried out with the support of a vector that delivers the transgene. The introduction of a gene in the form of naked DNA into the system or even directly into the target organ presents limited effectiveness due to a fast degradation of nucleic acid.\(^1\)\(^7\)

**The target cell: the hepatocyte**

The third element in gene transfer is the target cell, which allows transcription and expression of the gene. A transgene can be delivered into the hepatocyte according to two different methods: *in vitro-in vivo* and *in vivo*.

**Gene transfer methods**

**In vitro-in vivo method**

In this method, the hepatocytes are extracted by dissociation of a part of partial hepatectomy, or of the liver of a donor animal. Then, the hepatocytes are genetically modified immediately after a procedure using cellular culture. Subsequently, the genetically modified hepatocytes are introduced into the donor animal itself or into another animal. The earlier case characterizes an autologous transplantation of hepatocytes, whereas the latter case characterizes allogeneic or xenogeneic transplantation of hepatocytes. Genetic modification of hepatocytes in culture can be carried out using physical media or through retroviral vectors.\(^1\)\(^8\) Once genetically modified, the hepatocytes have to be reimplanted in a significant number into a site where their growth will be satisfactory. In this sense, the adaptation of cellular interactions, the existence of an extra-cellular matrix that favors growth, and the need for hepatotropic factors with portal origin favor the liver as the site for the definite implantation of genetically-modified hepatocytes. Hepatocytes are implanted into the liver by a catheter inserted into the portal vein (or into a tributary vein) or by intraspleen administration, which is followed by the migration of these hepatocytes into the liver. The feasibility of this method was successfully demonstrated by Chowdhury et al.\(^1\)\(^9\) with an animal model (Watanabe rabbit) for familial hypercholesterolemia, a disease caused by a genetic deficiency of low-density lipoprotein receptors (LDLRs). Unsuccessful results presented by a pilot study of liver-directed gene therapy carried out with five familial hypercholesterolemia patients - transgene expression in a limited number of hepatocytes - indicated the need for improved efficacy of phenotypic correction of familial hypercholesterolemia by the *in vitro-in vivo* method.\(^2\)\(^0\)\(^2\)\(^1\)

The main limitation of this method for its application in humans is the number of hepatocytes engrafted. This limitation could be overcome with cell banking of allogenic hepatocytes.\(^2\)\(^2\) Optimal gene transfer rates into hepatocytes of fetuses can be obtained with retroviral vectors (as high as 90\%). In addition, cryopreservation, including long-term cryopreservation (8 months), had little effect on cell viability and on the transduction of these hepatocytes by retroviral vectors. The viability of thawed hepatocytes remained high (75-85\%), and the transduction was identical to that of freshly isolated cells.\(^2\)\(^3\) In order to obtain optimal *in vitro-in vivo* results, in turn, it is possible that the delivery of a positive selective advantage to genetically modified hepatocytes could increase their ability to repopulate the liver in relation to native hepatocytes.\(^2\)\(^0\)\(^2\)\(^3\) Although this method consists of an autologous transplantation of hepatocytes, it does not, in theory, prevent immunization of the recipient against the foreign protein produced by genetically modified hepatocytes.

**In vivo method**

The *in vivo* method is aimed at being more efficient than the *in vitro-in vivo* method in relation to the number of genetically modified hepatocytes. The method consists of administering the vector carrying the transgene directly into the hepatic parenchyma, the biliary tract, the portal vein, or the hepatic artery.\(^1\)\(^6\)\(^2\)\(^4\)\(^2\)\(^6\) Portal infusion is currently the most widely used technique.

When using *in vivo* retroviral vectors, it is important to consider that, first, gene transfer should be carried out during hepatic regeneration, which is more frequently induced by partial hepatectomy; second, that extrahepatic dissemination of retroviruses may cause the contamination of the germinal layer. In order to avoid or reduce dissemination, surgical procedures could be used to remove the liver, which would then be genetically modified *ex vivo* with retroviral vectors perfused into an isolated perfused liver, and, next, an autologous, orthotopic transplantation of the genetically modified liver would be carried out.\(^2\)\(^7\)
Also, in order to avoid ex vivo perfusion procedures in an isolated perfused liver, and to avoid orthotopic transplantation of the liver, the in vivo-in situ perfusion method allows temporary hepatic vascular exclusion from the splanchnic and systemic circulation during the infusion of retroviral vectors, thus avoiding any extrahepatic dissemination of these vectors.28

**The vectors**

Different types of vectors have been described in the literature, and they have been divided into nonviral and viral vectors.

**Nonviral vectors**

Among nonviral vectors that allow transfection of hepatocytes in vivo or in vitro, there are physical and chemical methods, such as the use of calcium phosphate precipitates,29 dextran,30 particle bombardment,31 and liposome.32 The efficacy of nonviral vectors, despite presenting a tendency to improve, remains inferior to that of viral vectors.

**Viral vectors**

Different viruses have been described for gene transfer into hepatocytes, such as adenovirus-associated vectors (AAV), lentiviral vectors, such as human immunodeficiency virus (HIV), and baculovirus.33 These viral vectors present the advantage of being able to transduce cells independently of the host's cell cycle phase. Two other types of viral vectors, however, have been specifically studied in the last few years: the adenoviral vectors and the retroviral vectors.

**Adenoviral vectors**

Currently, 10% of clinical protocols employ adenoviral vectors.34 The advantages of adenoviral vectors are the following: (i) obtainment of high-titer viral particles (1012 ffu/ml); (ii) transduction of cells independently of target-cell cycle phase(35); (iii) obtainment of hepatic tropism with 95 to 100% efficiency of hepatocyte transduction after systemic infusion36 or biliary tract infusion;26 (iv) use of adenovirus as a live attenuated virus oral vaccine, as it has been administered to US Army troops for over 20 years without any negative consequences, especially concerning risk for cancer;37 (v) use of incorporated sequences of DNA that can be as extensive as those introduced into retroviral vectors.

The disadvantages of adenoviral vectors, in turn, are the following: (i) the transgene expression is transient or relatively momentary; (ii) the episomal characteristic of the adenovirus is partially responsible for this temporary expression; (iii) tissue transduction by systemic infusion is nonspecific - a great number of cellular types can express adenovirus receptors and, thus, it is difficult to transduce one type of organ without partially transducing other types of organs; (iv) direct cytotoxic reactions caused by in vivo administration of adenovirus. Currently, cytotoxic reactions represent the most significant disadvantage caused by adenoviral vectors and significantly limit its use. Intravascular or biliary administration are optimal procedures for liver transduction, since 95 to 100% of the hepatocytes are transduced.26,36 The transgene expression, however, is described as reducing significantly 1 week after its administration. After 3 to 4 weeks, the transgene expression is not present. Actually, a cellular immune response is stimulated and leads to the destruction of genetically modified hepatocytes, massive hepatitis, and repopulation of the liver with nontransduced hepatocytes.38,39 It is possible that the adenoviral vector induces a direct cytotoxic reaction due to expression of viral proteins. The extinction of transduced cells, however, would be a result of cellular or humoral immunity to protein-encoding transgene.40 At last, a specific humoral reaction against capsid proteins can render all in vivo readministration of adenoviral vectors ineffective.

**Retroviral vectors**

Currently, 60% of clinical protocols in gene therapy employ retroviral vectors.34 The most widely used retroviral vectors are derived from the Moloney murine leukemia type C virus.41 Defective retroviral vectors (which do not contain necessary genetic information for an infectious cycle) are produced by encapsulation cells. The viral genes gag, pol, and env have been completely deleted. The eliminated DNA is replaced with a marker gene or therapeutic transgene usually associated with a selection gene, for example, the Escherichia coli beta-galactosidase gene and the puromycin resistance gene, respectively. These vectors present cloning abilities of approximately 8 kb.41

The advantages of using retroviral vectors are the following: (i) orderly integration of the viral genome into target cell’s genome, which allows for a long-term, stable expression of the transgene, even if the transduced cell gets divided - this is a favorable factor for using retroviral vectors in the treatment of metabolic diseases in children;42,43 (ii) if defective, retroviral vectors can infect only one target cell; (iii) once they are integrated into the genomic DNA, defective retroviral vectors cannot produce viral particles inside these cells, and the retroviral cycle is interrupted at the stage of proviral integration; (iv) relative absence of immunologic reaction after direct injection of the recombinant retrovirus or after transplantation of autologous cells transduced in vitro.

The disadvantages of retroviral vectors are the following: (i) obtainment of lower-titer retroviral particles (108 ffu/ml) in relation to adenoviral vectors - in fact, the efficiency of transduction is connected to the number of infectious particles/number of target cells to be transduced ratio, as recently demonstrated by Kitten et al.44 and De Godoy et al.;45 (ii) a nonspecific integration of the retroviral vector
into the genome of the target cell may occur in a tumor suppressant gene or in a site susceptible to activating an oncogene;\(^{46}\) (iii) gene transfer by retrovirus vectors occurs only in cells actively replicating their DNA;\(^{47}\) since the transduction depends on the entry of the retroviral DNA-protein complex from the cytoplasm into the nucleus of the target cell - the entry of the retroviral DNA-protein complex into the nucleus and its integration into the host’s cell genome in the form of provirus depends on the nuclear envelope breakdown during mitosis.\(^{48}\) This is a significant limitation for direct in vivo transduction, since few types of tissue or cells actively suffer division under physiological conditions, including hepatocytes of a normal liver.

In order to overcome this limitation, in vivo transduction of hepatocytes can be carried out during hepatic regeneration previously induced by partial hepatectomy, for example.

**Liver regeneration**

The liver is an extremely complex organ with over 5,000 functions. It has two different types of vascularization and several types of cell. The ability of the liver to regenerate is not well understood yet from an ontogenic point of view. Liver regeneration is even more unique if we consider that all hepatic functions that are essential for homeostasis are not well understood yet from an ontogenic point of view.

Liver regeneration is even more unique if we consider that, while the liver has two different types of vascularization and several types of cell, the ability of the liver to regenerate is not well understood yet from an ontogenic point of view. Liver regeneration is even more unique if we consider that, while the liver has two different types of vascularization and several types of cell, the ability of the liver to regenerate is not well understood yet from an ontogenic point of view.

Liver regeneration

The liver is an extremely complex organ with over 5,000 functions. It has two different types of vascularization and several types of cell. The ability of the liver to regenerate is not well understood yet from an ontogenic point of view. Liver regeneration is even more unique if we consider that all hepatic functions that are essential for homeostasis are maintained during liver development;\(^{49-51}\) and that, while being in ex vivo conditions, during the S phase of the hepatocyte cell cycle in isolated perfused rat liver (normothermal conditions), it progresses in a similar way - timing, quantity, and duration of DNA synthesis, as well as in terms of lobular distribution - to in vivo liver regeneration after partial hepatectomy.\(^{27}\)

The normal adult liver presents minimum replicating activity. In a normal liver, the adult hepatocyte is a quiescent cell, with a life span of 200 to 400 days.\(^{52}\) Normal livers also present 0.2 to 0.5% hepatocytes in the DNA-synthesizing phase\(^{53}\) - in other words, one mitosis for 20,000 hepatocytes.\(^{54}\)

Yet, the liver is an organ that can, almost immediately, start a process of regeneration in response to a cell loss caused by toxic, physical, or infectious lesion. The most widely used experimental model to study liver regeneration in rats is partial hepatectomy (2/3), described in 1931 by Higgins & Anderson.\(^{55}\) After partial hepatectomy, the resected lobes will not grow again, and it is possible to verify hyperplasia of the residual lobes in order to reestablish the ideal hepatic cell mass in relation to body weight. The cells that proliferate are hepatocytes, bile duct epithelial cells, endothelial cells, Kupffer cells, and Ito cells. Depending on the kinetics of DNA synthesis through these different cells, they can also be transduced by retroviral vectors. For example, we may consider bile duct epithelial cells, which can be transduced with retroviral vectors containing the \(cfr\) gene in patients with cystic fibrosis. In this case, approximately 7 to 10 days after partial hepatectomy in rats, the liver recovered its normal mass.\(^{56}\)

Liver regeneration induced by partial hepatectomy presents a precise onset, and it does not cause any lesion to residual lobes. Also, the kinetics of DNA synthesis by hepatocytes have been widely studied. The S phase peak in hepatocyte cell cycles can occur 24 hours after partial hepatectomy, at which point 35% of hepatocytes are synthesizing DNA.\(^{57-59}\) Bile cell replication occurs 24 hours after the hepatocyte cell cycle S phase peak. Since only 2/3 of the liver is resected, restoration of the original number of hepatocytes is, in theory, obtained with 1.66 cell cycles per residual hepatocyte.\(^{51}\)

Moreover, in the deficient liver, partial hepatectomy is an actual limitation for gene transfer. The resection of liver mass may unbalance the homeostasis, which is probably already inconsistent in the deficient liver. Investigation of mitogenic properties of the liver may result in an alternative for the induction of liver cell proliferation in vivo without having to resort to a previous partial hepatectomy. In this sense, the method could be used to improve in vivo gene transfer into the liver using retroviral vectors.

**Growth factors and gene transfer**

Mitogenic growth factors are described as substances capable of inducing DNA synthesis and mitosis of hepatocytes in a serum-free cell culture medium. Several substances have been described as having these characteristics, such as EGF (epidermal growth factor),\(^{60}\) TGF-\(\alpha\) (transforming growth factor-\(\alpha\)),\(^{51,56}\) KGF (keratinocyte growth factor),\(^{51}\) and, especially, HGF (hepatocyte growth factor).\(^{62}\) HGF is the most powerful mitogen known for the liver.\(^{56,62-64}\) HGF has not been reported as presenting any risk of cancer, conversely to TGF-\(\alpha\)\(^{65}\) and KGF.\(^{66}\) Recently, hepatocyte transduction rates of 30% were obtained with continuous infusion of recombinant HGF simultaneously with infusion of retrovirus vectors into the portal vein of mice.\(^{67,68}\)

**Conclusion**

The introduction of a gene into a target cell, namely a hepatocyte, is only the first step in somatic gene transfer. Besides the introduction of the gene, there are other obstacles that need to be overcome. The transferred gene has to endure the whole life span of the genetically modified cell. Moreover, the transferred gene has to be transmitted to the genome of cells originated by subsequent cell division. In this sense, in the search for a clinical treatment for inborn metabolic disease of the liver in children, it is still necessary to obtain a long-term stable transgene expression at adequate levels.
Acknowledgment

We thank Professor Nelson Augusto Rosário Filho for his invaluable contribution.

References

Gene transfer as treatment for metabolic inherited liver diseases - Godoy LG


47. Miller D, Adam M, Miller A. Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 1990;10:4239-42.


Correspondence:
Dr. José Luiz de Godoy
Disc. de Cirurgia Pediátrica – Hospital de Clínicas – UFPR
Rua General Carneiro, 181 – 13º andar
CEP 80060-900 – Curitiba, PR, Brazil
Phone: + 55 41 360.1870 – Fax: + 55 41 264.5872
E-mail: jlgodoy@hc.ufpr.br