Peritoneal Dialysis in the 21st Century: The Potential of Gene Therapy

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Abstract. One of the greatest biotechnologic advances of the last 25 yr is genetic engineering—the ability to identify and isolate individual genes and transfer genetic elements between cells. Genetic engineering forms the basis of a unique biotechnology platform called gene therapy: an approach to treating disease through genetic manipulation. It is becoming clear that during peritoneal dialysis, the peritoneal membrane undergoes various structural and functional changes that compromise the dialyzing efficiency of the membrane and eventually lead to membrane failure. A gene therapy strategy based on genetic modification of the peritoneal membrane could improve the practice of peritoneal dialysis through the production of proteins that would be of therapeutic value in preventing membrane damage and preserving its dialyzing capacity. The peritoneal membrane can be genetically modified by either ex vivo or in vivo gene transfer strategies with a variety of potentially therapeutic genes, including those for anti-inflammatory cytokines, fibrinolytic factors, and antifibrotic molecules. These genes could be administered either on an acute basis, such as in response to peritonitis, or on an intermittent basis to maintain physiologic homeostasis and perhaps to prevent the adverse changes in the membrane that occur over time. The anticipated effect of a gene therapy strategy could be measured in maintenance of desired transport characteristics and in patients being able to remain on the therapy for longer periods of time without the negative outcomes. In summary, the use of a gene therapy strategy to enhance peritoneal dialysis is an innovative and exciting concept with the potential to provide new treatment platforms for patients with end-stage renal disease.

One of the greatest biotechnologic advances of the last 25 yr is genetic engineering—the ability to identify and isolate individual genes and transfer genetic elements between cells. This technology, combined with the rapid progress toward mapping the human genome and the emerging field of bioinformatics, opens the door to identifying genes involved in a variety of disease processes and manipulating the genetic makeup to correct or restore normal function to cells. Genetic engineering forms the basis of a unique biotechnology platform called gene therapy: an approach to treating disease through genetic manipulation, a strategy that will certainly change the face of medicine in the 21st century.

Gene Therapy

Gene therapy is the treatment of a disease or chronic condition through the introduction of genetic elements into the somatic cells of the patient resulting in a change in gene expression to produce a desired therapeutic effect. Gene therapy was originally proposed in the late 1980s as a treatment strategy for diseases caused by single gene defects, such as cystic fibrosis (1). It was thought, rather naively and optimistically at the time, that replacement of a damaged or nonfunctioning gene with a normal gene copy would lead to correction of the condition. Because the molecular tools for genetic manipulation were available, it seemed like a guaranteed strategy. The first human gene therapy experiment was initiated in September 1990, for the treatment of a rare congenital immunodeficiency disorder called adenosine deaminase deficiency (2).

The 10 yr since the first patients were treated have not lived up to the initial outlook and high expectations. The “cure” was not immediate. There were problems with gene delivery, failure to achieve therapeutic levels of transgene expression, and at least one death directly attributed to gene delivery methodology (3). With these setbacks came the realization that diseases are complex multigene phenomena, that more basic research is required before moving to the clinic, and that gene therapy clinical trials must be more rigorously controlled. Enthusiasm for gene therapy may have wavered, but research now continues armed with more realistic expectations. The recent report of full correction of severe combined immunodeficiency X1 disease in four infants by a gene therapy approach has been very welcome news indeed (4).

Currently, there are 425 gene therapy trials worldwide, involving more than 3400 patients (5). Most of these have been for the treatment of cancer, but many have been treated for monogenic and infectious diseases, as well as several chronic conditions. We have previously addressed the use of gene therapy to enhance peritoneal dialysis (PD) (6,7). These comprehensive reviews have covered methods of genetic modification, peritoneal membrane biology, and gene transfer to the peritoneal mesothelial cell and the peritoneal cavity. Here, we will focus on the clinical applications of gene therapy for PD.
Application of Gene Therapy to PD

How might a gene therapy strategy be applied to PD? As with any proposed therapeutic approach, the initial step is to identify the condition to treat. It is becoming quite clear that the peritoneal membrane undergoes various structural and functional changes with time on dialysis (8,9). These alterations include thickening of the submesothelial layer, deposition of collagen and extracellular matrix components, decreased fibrinolytic capacity, neoangiogenesis and vasculopathy of the peritoneal microvasculature, increased deposition of advanced glycation end products, and increased membrane permeability (10–13). These changes may compromise the dialyzing efficiency of the membrane and eventually lead to membrane failure, precluding continuation of this mode of therapy.

The goal of a gene therapy strategy for PD is straightforward: to modify the peritoneal membrane or other resident cells of the peritoneal cavity to preserve the overall structure and function of the membrane, and maintain or preferably enhance dialyzing capacity. In this manner, gene therapy could be designed to prevent the fibrotic changes that can occur in the membrane with time on PD, to improve a patient’s transport status, or to prevent oxygen radical–mediated damage to the membrane. Some basic questions must be taken into consideration in prescribing a specific gene therapy regimen. First, the pathologic condition or conditions and the specific goal of gene therapy must be identified. Will the therapy provide for the expression of a particular factor, or will it prevent its expression? What is the target cell for genetic modification, and what gene delivery agent is most appropriate? Will gene therapy require single or repeated gene transfers? And how will transgene expression from modified cells be controlled?

Gene therapy for PD can be structured to address both acute and chronic conditions. These areas, and the genes that may be applicable as molecular therapeutics, are listed in Table 1.

Gene Therapy for Peritoneal Inflammation

Gene therapy could be used to protect the membrane from acute inflammation and injury resulting from peritonitis or chronic inflammation due to prolonged exposure to solutions. Modification of peritoneal mesothelial cells or peritoneal leukocytes with the genes for anti-inflammatory factors may be effective in moderating the inflammatory response and decreasing cellular damage from activated polymorphonuclear leukocytes (PMN) and macrophages. Potential anti-inflammatory therapeutics include the interleukin-1 (IL-1) receptor antagonist (IL-1RA), the soluble receptor to tumor necrosis factor alpha, and IL-10. The strategy of downregulating inflammatory pathways through blocking the action of transcription factor nuclear factor kappa B (NFκB) is currently under evaluation (14,15). Because inflammation can lead to the release of oxygen radicals that are potentially damaging to the membrane, genetic modification with the gene for the antioxidant enzyme catalase may confer increased resistance to oxygen free radical–mediated cytotoxicity (16).

As an example of how gene therapy could be used for peritoneal inflammation, we recently generated rat peritoneal mesothelial cell clones stably expressing human IL-1RA (huIL-1RA), and assessed the sensitivity of these clones to activation by IL-1β. Recombinant IL-1RA protein production in these clones ranged from 4 to 70 ng IL-1RA/10⁶ cells per day and reflected the relative amounts of IL-1RA specific mRNA detected in the individual clones (Figure 1). IL-1RA–producing clones were less sensitive to activation by IL-1β, as evidenced by significantly decreased levels of monocyte chemoattractant protein-1 produced in response to increasing levels of IL-1β as compared with control, or unmodified, cells (Figure 2). These findings suggest that increasing the anti-inflammatory potential of the peritoneal membrane through genetic modification of mesothelial cells with a factor such as IL-1RA may be effective in blunting the response of mesothelial cells to proinflammatory mediators and in moderating the effects of inflammation on the peritoneal membrane. The potential of IL-1RA as a therapeutic intervention is under investigation in a number of disease states, including its use in ameliorating complications of renal disease (17).

Gene Therapy to Maintain Membrane Integrity

Gene therapy can also be used to restore to normal levels activities of the membrane that become compromised during dialysis or peritonitis. Increasing the tPA/PAI-1 ratio through the transgene-mediated production of tissue plasminogen acti-

Table 1. Gene therapy for peritoneal dialysis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Potential Therapeutic*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>IL-10, IL-1RA, sTNFrp55, NFκB antisense</td>
</tr>
<tr>
<td>Preservation of membrane integrity</td>
<td>tPA, uPA, PAI-1 antisense thrombomodulin catalase</td>
</tr>
<tr>
<td>increase fibrinolytic capacity</td>
<td>HAS, VEGF antagonists</td>
</tr>
<tr>
<td>increase anticoagulant capacity</td>
<td>TGF-β antagonists (e.g., decorin), HSP47 (inhibition of collagen deposition)</td>
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* IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; sTNFrp55, soluble TNF receptor (55 KD); NFκB, nuclear factor kappa B; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor–1; HAS, hyaluronan synthase; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor beta; HSP47, heat shock protein 47.
Gene Therapy for Peritoneal Fibrosis

Fibrotic changes in the membrane become more apparent with time on PD. Transforming growth factor beta (TGF-β) may play a major role in these changes because it is activated by high glucose and inflammation, found in increased levels in continuous ambulatory PD patients (21), and in turn upregulates the synthesis of a number of factors, including vascular endothelial growth factor, extracellular matrix (ECM) components, and PAI-1. Strategies to intervene in TGF-β–mediated pathways include transfer of gene for the proteoglycans decorin (22), antiangiogenic factors to counteract vascular endothelial growth factor–mediated effects (23,24), and the inhibition of NFκB (14,15), a transcription factor that governs TGF-β transcription as well as transcription of a number of proinflammatory cytokines. Inhibition of TGF-β expression by antisense oligonucleotides was shown to suppress ECM accumulation in experimental glomerulonephritis (25) and may be effective in this application as well. It has recently been hypothesized that antisense mediated inhibition of the heat shock protein 47 may be effective in regulating intraperitoneal collagen deposition and may therefore have a therapeutic effect in prevention of fibrotic syndromes (26).

Cellular Targets for Gene Delivery

We have advocated that the peritoneal mesothelial cell be the primary target of gene transfer to the peritoneal cavity (6,7). Mesothelial cells, by virtue of their location on all visceral and parietal surfaces of the peritoneal cavity, constitute a large target cell population and actively participate in a number of physiologic processes in the peritoneal cavity, including response to inflammation, fibrinolysis, and wound healing and tissue repair. Genetic intervention in these pathways through gene transfer could be an effective way of modulating physiologic or pathophysiologic events in the PD patient. In addition, the mesothelial cell can be modified to produce factors that not only affect endogenous gene expression, but also the activities of surrounding cells through secretion of factors into the peritoneal lumen or underlying tissue.

The mesothelial cell is not the only target for genetic modification in the peritoneal cavity, however. Peritoneal macrophages, neutrophils, lymphocytes, fibroblasts, and endothelial cells all are important players in host defense, inflammation,
fibrosis, and peritoneal transport, and should be equally considered for genetic modification depending on the ultimate goal of the gene therapy regimen.

**Strategies for Genetic Modification**

Genetic modification can be accomplished through one of two strategies (Figure 3). In *ex vivo* gene transfer, the target cells are isolated from the patient, genetically modified in culture, and then reimplanted back into the patient. In *in vivo* gene transfer, the genetic elements are delivered directly to the patient and genetic modification occurs *in situ*.

Peritoneal mesothelial cells are an excellent choice for *ex vivo* gene therapy (Figure 4). They can be isolated from omentum and grown in culture (27), genetically modified by a variety of gene delivery agents (6), and reimplanted back into the peritoneal cavity (28, 29). Mesothelial cells isolated from the patient at the time of catheter implantation would provide a population of cells in good health (*i.e.*, predialysis). They could be expanded and immediately stored in liquid nitrogen for future use, generating a repository of the patient’s own cells, or genetically modified before freezing (Figure 4). This service could be performed in hospital or medial facility with services for cell culture and gene transfer.

The stored cells would then be available for infusion as needed; this could be in response to an acute condition such as peritonitis, or perhaps on an intermittent, prophylactic basis during ongoing dialysis to maintain the peritoneal levels of various factors (*e.g.*, fibrinolytic enzymes) within normal physiologic range. Cells would be infused into the peritoneal cavity through the indwelling catheter in such a volume that enough fluid remains in the peritoneal cavity to keep surfaces wet but will allow cells to attach themselves to peritoneal surfaces. Studies in rats (30), rabbits (28), and humans (28) have indicated that mesothelial cells will attach on a damaged or irritated membrane—for example, on a membrane after peritonitis or after chronic exposure to dialysis solutions. Further studies may be required to define the conditions best suited for mesothelial cell reimplantation in a dialysis patient after injury.

There are several advantages to *ex vivo* gene therapy. Because genetic modification is done in culture, the cell population can be well characterized before implantation. Simple infusion of mesothelial cells after injury may allow remesothelialization of the membrane to occur faster than it would on its own. In addition, reseeding the membrane with genetically modified cells could confer an added property to the membrane—perhaps a function that speeds the healing process or reestablishment of a protein that is decreased during PD. Finally, implantation of permanently modified cells will create a membrane with stable transgene expression as the cells divide and pass the transgene to the subsequent generations.

**In Vivo Gene Therapy**

*In vivo* gene therapy is a much simpler strategy than *ex vivo* and involves direct delivery of the gene to the peritoneal cavity. In *ex vivo* gene therapy, we focused on the genetically modified mesothelial cell. In *in vivo* gene therapy, the target cells are theoretically all of the resident cells in the peritoneal cavity, including the mesothelial cells, the peritoneal macrophages, and PMN. The *in vivo* gene therapy strategy therefore must first identify the cellular target and then identify the best delivery agent for gene transfer to those cells.

Figure 3. Ex vivo and in vivo gene transfer strategies. In *ex vivo* gene transfer, the cells of interest are removed from the patient and established in cell culture. They are genetically modified by an appropriate gene delivery agent (*e.g.*, naked DNA, liposomes, recombinant viruses) and then infused back into the patient. In *in vivo* gene transfer, the genetic material is delivered directly to the patient and genetic modification takes place *in situ*. 
Gene Delivery Agents

Gene delivery agents are categorized either as viral or nonviral based. Viral systems are based on replication deficient, recombinant viruses in which virus sequences are replaced with sequences of the desired foreign genes. Systems currently in use are based on adenovirus, adeno-associated virus, retrovirus, herpes, and lentivirus vectors.

The viral gene transfer system most often used for intraperitoneal delivery is based on the recombinant adenovirus. Adenovirus efficiently infects mesothelial cells and produces high levels of transgene product throughout the peritoneal cavity (31). It has been used extensively for preclinical animal studies (32,33) and in clinical trials for abdominal carcinomas (34–36). However, because adenovirus is not cell type specific, it infects not only the mesothelial cells, but also the resident PMN and macrophages. Although it is an excellent model delivery system, its immunogenic properties preclude readministration and would probably rule it out as the delivery agent of choice in this specific application.

In systems based on nonviral delivery agents, genetic material can be delivered as naked DNA or encapsulated by a variety of materials (e.g., lipids, polyamions, albumin) before delivery. The genetic elements best delivered by this approach are antisense oligonucleotides, short nucleic acid sequences designed to block RNA transcription, translation, or both, effectively preventing specific endogenous expression. The nonviral gene delivery agents have not been shown to be efficient in gene transfer to normal mesothelial cells but are quite effective in delivery to peritoneal carcinomas and metastases (37–39), as well as other cell types, specifically the peritoneal macrophage. Liposomes and microspheres (15,40) efficiently deliver antisense oligos and specific antibodies to macrophages, for example, providing a nice system for selective genetic modification of these cells.

The choice of delivery agent and genetic element may be dependent on timing and the condition to be addressed. For acute conditions such as peritonitis, it would be preferable to have a therapeutic that could be administered quickly and was effective immediately. Antisense-oligonucleotide–mediated gene therapy may produce a more immediate effect than delivery of a cDNA for a therapeutic gene, where the gene would have to be transcribed, the protein translated, and the protein secreted and accumulate in critical amounts to achieve a therapeutic goal. It must be kept in mind that antisense-based therapy results in inhibition of targeted gene expression, whereas transfer of a cDNA results in the production of a specific factor. These genetic approaches are in contrast to another option: direct delivery of the therapeutic protein itself. The benefits of direct delivery (i.e., immediate availability of the protein) would have to be considered in the light of short half-life of most recombinant proteins and, if delivered locally,
into the peritoneal cavity, perhaps in the dialysis solution, the amount needed to achieve a therapeutic threshold.

**Regulation of Transgene Expression**

An issue central to gene therapy is the control of transgene expression once the gene or genes are in the cell. Whether the therapeutic strategy involves the modification of a normal cellular function (i.e., inflammation) or provides for a new one, transgene expression must be tightly controlled to achieve the desired therapeutic end. Systems have been designed to provide regulation of transgene expression by small-molecule drugs, resulting in pharmacologic regulation of gene expression (41), or by physiologic stimuli (42). This approach may be necessary when cells are permanently genetically altered. In cases where expression is transient (i.e., nonchromosomal integration of the delivery vector or the use of antisense oligos), or stability of the transferred elements is limited, gene expression may be relatively transient and without a need for pharmacologic control. In this case, the desired therapeutic effect may be achieved through repeated administration of the genetic elements.

**Gene Therapy–Based Prescriptions for the PD Patient**

We will now describe two situations in which a nephrologist might prescribe gene therapy for a PD patient: first, as an appropriate response to an acute condition, such as peritonitis; and second, as ongoing intermittent therapy for chronic conditions.

**Gene Therapy for Peritonitis**

Upon diagnosis of peritonitis, the PD patient would receive an appropriate course of antibiotics and a gene therapy prescription. The goals of gene therapy are twofold: to deactivate peritoneal macrophages and blunt the inflammatory response; and to reseed the membrane and restore its antioxidative and fibrinolytic properties. The gene therapy prescription would have two components: delivery of antisense oligos for *in vivo* targeting of the peritoneal macrophages and delivery of genetically modified mesothelial cells for *ex vivo* gene transfer.

Macrophages would be selectively targeted with microencapsulated antisense oligos to NFκB or to TGF-β. Inhibition of NFκB expression could blunt the production of proinflammatory cytokines by the macrophages; selective inhibition of macrophage-produced TGF-β may prevent activation of TGF-β–mediated downstream pathways and limit profibrotic events, including ECM deposition, fibroblast proliferation, and increase in vessel permeability and angiogenesis. As the effect of the antisense oligos is relatively short-lived (24 to 48 h), they can be used to transiently block an action for therapeutic benefit without permanent genetic alterations.

The mesothelial cells for infusion would be modified with the genes for catalase and tPA; this could be done immediately before implantation or, preferably, after the initial isolation at the time of catheter implantation (Figure 4). Genetic modification of the cells with catalase and tPA is designed to reduce oxygen radical–mediated damage caused by activated PMN and to increase the fibrinolytic capacity of the membrane, respectively. The number of infused cells might be determined by the severity of peritonitis and inflammation, possibly measured by peritoneal levels of proinflammatory cytokines. In addition, these cells could be either transiently or permanently modified. Transient modification would provide a short-term effect and then leave a membrane with normal characteristics. Permanent modification would produce a membrane that retains these characteristics, with the altered cells increasing in number with each cell division. Transgene production would be controlled through the use of a gene promoter that is activated upon inflammation, or by a small molecule drug, giving the nephrologist the ability to “prescribe” the correct dose of gene product for the patient.

The timing of this therapy may be such that the antibiotics and encapsulated oligos would be delivered upon diagnosis of peritonitis and the mesothelial cells delivered a day or two later when the acute phase is ending. The anticipated net effect of this gene therapy prescription would be to decrease the proinflammatory response, repopulate the mesothelial cell monolayer after peritonitis, reduce oxygen radical–mediated damage, decrease fibrin deposition, and facilitate membrane recovery by return of transport parameters to normal values. Success of this treatment would be monitored by the speed with which transport returns to normal and by the membranes’ long-term performance.

**Gene Therapy for Chronic Conditions**

Gene therapy can be used for treatment of chronic conditions as well. There are a number of changes that occur in the membrane of long-term PD patients: collagen and ECM deposition and membrane thickening, advanced glycation end product deposition, vasculopathy and angiogenesis, and changes in membrane permeability and transport. It may be possible to prevent or mediate the onset or development of changes through ongoing or prophylactic gene therapy. Mesothelial cell–mediated *ex vivo* gene transfer, by means of cells genetically modified with decorin to inhibit TGF-β, for example, may be effective, as might be the transfer of antiangiogenic factors to counteract new vessel development and the increased permeability of the vessels themselves. Infusion of modified cells every 1 or 2 mo might be enough to prevent membrane thickening or to maintain transport parameters. *In vivo* gene therapy to the peritoneal macrophage might also be an option. Delivery of encapsulated antisense oligos to TGF-β on an intermittent basis could keep the peritoneal expression of TGF-β at near-normal levels.

**Gene Therapy Perspective**

How close are we to gene therapy for PD? There is a considerable amount of ongoing research in gene transfer to the peritoneal mesothelial cell, to macrophages, and to the peritoneal cavity with promising results in animal models (18,30,31,43–45). Once a therapeutic effect is shown in models of PD or peritoneal fibrosis, such as a reduction in mem-
brane pathology or preservation of membrane transport, we foresee clinical trials within 5 yr.

For gene therapy for PD to become a reality a number of issues must fall into place. Research must identify gene transfer agent or agents that would provide not only for targeted in vivo delivery to the resident cells of the peritoneal cavity, but for repeated administration as well. Nephrologists and research scientists in PD must join their colleagues in other medical disciplines in using genomics and bioinformatics to determine if correlations exist between gene expression in peritoneal tissues and development of chronic and acute conditions. This knowledge may provide a slate of genes that may be potentially therapeutic in maintaining membrane viability or that may be potentially therapeutic in preventing the onset of conditions that predispose patients to membrane failure. And finally, and perhaps most important, nephrologists must expand their expectations of new therapies beyond the traditional areas such as new dialysis solutions and be willing to change their current practices to incorporate new technologies such as gene therapy into a comprehensive plan of care for the patient with end-stage renal disease.

Summary

There are significant rewards for continuing to push the concept of gene therapy for PD forward. The preclinical research that must be carried out to identify and test genes in PD-relevant models should provide an in-depth study of the biology of the peritoneal membrane and the molecular consequences of exposure to PD solutions and peritonitis. This, in and of itself, should facilitate the identification of new treatment strategies. The gene therapy strategies that we have suggested in this review will, we hope, result in preserving the membrane: Potential for improving peritoneal dialysis through gene therapy. Semin Dial 11: 218–227, 1998


References


29. Hekking LHP, Harvey VS, Havenith KEG, Beelen RHJ, Jackman RW, Nagy JA: Genetically-modified mesothelial cells as a tool to visualize mesothelial cell damage in models of acute inflammation and chronic peritoneal dialysis. *Perit Dial Int* 20[Suppl 1]: S9, 2000


40. Hekking LHP, Harvey VS, Havenith KEG, Beelen RHJ, Jackman RW, Nagy JA: Genetically-modified mesothelial cells as a tool to visualize mesothelial cell damage in models of acute inflammation and chronic peritoneal dialysis. *Perit Dial Int* 20[Suppl 1]: S9, 2000

