

Gene Therapy for Peripheral Arterial Disease and Nursing Implications: Clinical Experience on the use of Sendai Viral Vector

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Abstract

Current trends in gene therapy affect nursing practice and research. As treatments with gene therapy progress, nurses are required to have advanced knowledge and techniques to provide nursing care for patients who undergo gene therapy. The challenges for nurses are adherence to ethical and regulatory guidelines, detailed knowledge of gene transfer and safety issues in the use of viral vectors, the ability to monitor unspecified symptoms, and the provision of non-standardized care for patients. In this article, the authors discuss implications for specialized nursing related to the use of virus vectors in gene therapy, with a focus on peripheral arterial disease.

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Introduction

As the field of gene therapy continues to evolve, nurses have experienced challenges in their roles. The role of a nurse in gene therapy is very important for patient safety, and also for coordination of the whole study of gene therapy. In many cases, research nurses are the ones who are directly involved with patients and administration of the therapy. The number of nurses employed as research nurses has reportedly increased, and research nurses are recognized as having an advanced, specialized nursing role [1]. The research nurses fully review and understand research protocols, assist with obtaining informed consent, provide information regarding the gene therapy to patients and interdisciplinary teams, coordinate treatment schedules, monitor adverse effects, and continuously assess patients' needs [2]. Despite over 2000 gene therapy clinical trials conducted worldwide, there are few articles related to gene therapy nursing. In this article, implications for advanced nursing concerning the use of gene therapy for peripheral arterial disease (PAD), with a focus on the Sendai virus (SeV) vector, are discussed.

Gene Therapy for Peripheral Arterial Disease

Gene therapy refers to the introduction of genetic materials (transgenes) into human subjects with the aim of modifying cells for the treatment of acquired disease [3]. To date, gene therapy research involving humans has been undertaken exclusively in patients who currently have few or no treatment options, and the therapy has come with high expectations. One of the diseases that is a focus for gene therapy is PAD, which includes a range of conditions affecting the arteries in the limbs caused by stenosis or occlusive atherosclerosis in a vascular bed [4]. PAD has become an important public health issue because of its high prevalence, affecting 12% of the adult population [5]. The clinical symptoms of PAD result from reduced blood flow to the legs, and it is often asymptomatic or observed as numbness in the early stage. As PAD progresses, however, patient quality of life decreases because of limited walking ability associated with pain in the lower legs during walking, which is called intermittent claudication (IC). If untreated, approximately one in four IC patients progresses to critical limb ischemia (CLI) within 5 years [6]. CLI induces pain at rest and ischemic ulcer/gangrene in the legs, and the mortality is as high as 20%, with major amputation required within 1 year in 40% of CLI patients [7]. Currently, there is no drug with proven efficacy for the treatment of CLI. Despite advanced techniques in surgical and endovascular treatment, a large number of patients are not suitable

for such therapy [8]. Therefore, every year, numerous patients are estimated to progress gradually to CLI, resulting in amputation. Poor prognosis and increasing immobility and mortality in patients with PAD have created a need for new alternative therapies to induce angiogenesis, with most emphasis being placed on gene therapy [9].

Because clinical training is conducted in a actual field of clinical practice, not a place intentionally created for students training, even the stress and pressure felt by the instructors, not to say the students, is enormous due to the unfrocking nature of any mistake even for the student during clinical practice training, however, in the simulation training the repeated learning is possible through the reproduction of the clinical situation and you can go through a trial and error while some mistakes are allowed without evoking any direct and deadly impact on the safety and rights of patients, even if you would commit them.

Gene Therapy for Peripheral Arterial Disease

Therapeutic angiogenesis modifies ischemic tissue to provide a proangiogenic environment through inducing growth of a capillary network [9]. Recombinant proteins, or genes encoding angiogenic growth factors, were used to enhance blood flow and expand the collateral circulation to ischemic tissue [4,8]. Since the end of the 1990s, gene therapy using therapeutic angiogenesis has been proposed as an innovative therapy for chronic arterial occlusion, and has become a clinically attractive potential alternative treatment option [10-12]. Up to the present time, the techniques of therapeutic angiogenesis using (1) recombinant protein (protein-based therapy), (2) gene delivery (gene therapy), and (3) bonemarrow/blood cells (cell therapy) have been clinically tested. Therapeutic angiogenesis for PAD has been demonstrated to induce neovascularization using many growth factors in preclinical studies [13]. Proangiogenic growth factors which have demonstrated angiogenic effects in PAD are vascular endothelial growth factor (VEGF), fibroblast growth

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factor (FGF), hepatocyte growth factor (HGF), hypoxia-inducible factor (HIF)-1, and developmentally-regulated endothelial locus (DEL)-1. The first-in-man clinical trial of therapeutic angiogenesis was conducted using naked plasmid VEGF₁₆₅ in 1994 [14]. Since then, the proof of concept in preclinical studies has shown promising results, yet few late-phase clinical trials have been performed [15].

Progress in Clinical Trials

There were 2076 gene therapy clinical trials approved worldwide in 36 countries between 1989 and 2014 [16]. The majority of gene therapy clinical trials (64.1%) aimed to treat cancer, and the indications of cardiovascular and ocular diseases accounted for only 9.4% of the trials [16]. In a meta-analysis of six randomized controlled trials, there was a significant clinical improvement compared with placebo in patients with PAD (odds ratio = 1.427; 95% confidence interval = 1.03–2.0; P = 0.033) [17,18]. Selected PAD gene therapy trials are shown in Table 1. One of the most promising angiogenic growth factors is FGF-2. The bulk of experimental data related to the prototypic FGF-2 have been obtained in an animal model *in vivo* to establish the potential for gene therapy trials in humans with PAD [19,20]. FGF belongs to a family of over 20 proteins. It was recognized as an angiogenic factor based on its induction

of endothelial cell proliferation, migration, and morphogenesis, extracellular matrix degradation, and vessel maturation [21]. A special feature reported for FGF-2 is its synergistic effects [22-25]. Therapeutic genes are transferred to targeted cells via vectors. There are viral vectors and nonviral vectors. The optimum carrier for FGF-2 to induce pronounced angiogenesis has also been studied, and it was found that recombinant SeV appeared to be the most efficient [26-28]. Intramuscular injection of SeV strongly boosted FGF-2, with the levels being as much as 300-fold higher than at baseline [29].

Clinical Experience of Sendai Virus Vector

The SeV-based vector has several theoretical and practical advantages over other viral vectors [30,31]. The SeV genome cannot integrate into the host genome, thereby reducing the risk for insertional mutagenesis [32,33]. This means that, unlike other viruses used in gene-transfer therapy vectors, SeV is a cytoplasmic negative-strand RNA virus that replicates entirely in the cytoplasm of cells and does not have a DNA intermediate. Moreover, SeV is not dependent on cell division for infection of the target cells, and requires only a brief contact time with cells to elicit cellular uptake. The recombinant SeV vector yields high expression levels of the transferred gene in a variety of cell and tissue types when compared with other virus vectors.

Angiogenic factors	Phase	Patients	No. of subjects (treated/controls)	Treatment	Vector	Primary endpoints	Outcome*
VEGF	I	CLI	9/-	pCK-VEGF ₁₆₅	Plasmid	Safety	Positive
VEGF	I	CLI	21/-	phVEGF ₁₆₅	Plasmid	Safety	Positive
VEGF	I	IC	15/3	adVEGF ₁₂₁	Adenovirus	Safety	Equivocal
VEGF	II	CLI	27/27	phVEGF ₁₆₅	Plasmid	Amputation	Negative
VEGF	II	IC	72/33	adVEGF ₁₂₁	Adenovirus	Peak walking time	Negative
VEGF	II	IC/CLI	18/17/19	adVEGF ₁₆₅ / phVEGF ₁₆₅	Adenovirus/ plasmid	Increased vascularity	Positive
HGF	I	CLI	78/26	pVAX1-HGF	Plasmid	Safety/TcPO ₂	Positive
HGF	I	CLI	22/-	pVAX1-HGF	Plasmid	Safety/ABI, ulcer size	Positive
HGF	I	CLI	21/-	pCK-HGF-X7	Plasmid	Safety and tolerability	Positive
HGF	II	CLI	21/6	VM202	Plasmid	Safety/ABI, VAS, ulcer	Positive
HGF	III	CLI	30/14	pVAX1-HGF	Plasmid	Rest pain, ulcer size	Positive
FGF-1	I	CLI	51/-	NV1FGF	Plasmid	Safety	Positive
FGF-1	II	CLI	51/56	NV1FGF	Plasmid	Ulcer healing	Negative
FGF-1	III	CLI	259/266	NV1FGF	Plasmid	Amputation or death	Negative
FGF-2	I	CLI	12/-	rSeV/df-hFGF-2	Sendai virus	Safety and tolerability	Positive
FGF-2	II	IC	127/63	rFGF2	Plasmid	Maximum walking time	Positive
HIF-1	I	CLI	34/7	Ad2/HIF-1α/VP16	Adenovirus	Safety and efficacy	Positive
HIF-1	II	CLI	213/76	Ad2/HIF-1α/VP16	Adenovirus	Maximum walking time	Negative
DEL-1	II	IC	52/53	VLTS-589	Plasmid	Maximum walking time	Negative

*Outcomes were evaluated for only primary endpoints and did not include any improvement in secondary endpoints.

Table 1: Selected PAD Gene Therapy Trials.

A new gene transfer vector based on a non-transmissible recombinant SeVvector carrying the FGF-2 gene (SeV/dF-hFGF2) was investigated in a first-in-man gene therapy clinical trial for CLI patients with no other option, and was completed in March 2011 [34]. A total of 12 patients, one limb per patient, were treated with SeV/dF-hFGF2 (product name DVC1-0101) in a four-dose escalation design. The cohort consisted of 10 males and 2 females (mean age 65 years), including 10 cases of arteriosclerosis obliterans and two cases of thromboangiitis obliterans. As a result of the study, it was proposed that DVC1-0101 was safe and well-tolerated, and significantly improved walking function [34].

Nursing implications for Gene Therapy

Adherence to Ethical and Regulatory guidelines

If the benefits of gene therapy were to outweigh the risks, then it would be ethically considered for non-option patients. In general, public opinion indicates approval for genetic technology when it is used for a specific purpose to cure, control, prevent, or advance the treatment of cancer or non-option diseases [35]. Therefore, the proposed gene-based therapeutic angiogenesis approaches would need to be rigorously assessed in a case-by-case basis.

The approval process for human gene therapy clinical trials is rigorous. It depends on the law and regulations in each country, and institutional, Food and Drug Administration, and National Institutes of Health approval are usually required. As for other clinical trials, gene therapy trials must also follow the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH), which provides an international quality standard for pharmaceutical product registration in Europe, Japan, and the United States. ICH guidelines have been adopted as law or ordinance for the performance of clinical trials in several countries. One of the guidelines, Good Clinical Practices (GCP), which has been agreed through the ICH process, describes the responsibilities and expectations of all participants in the conduct of clinical trials, including investigators, monitors, sponsors, and Institutional Review Boards (IRBs).

Informed Consent

Patients and their families need to be provided with opportunities to have their questions answered, and nurses are usually in a position to help educate patients and answer their questions [35]. The study protocol, clinical examination schedule, methodology of gene transfer, and consent forms are confusing and cumbersome. Informed consent is required not only at the beginning of the study but also whenever safety issues arise in relation to the gene therapy. Informed consent was required for a study of gene therapy using SeV at five time points as follows: 1) at the beginning of enrolment for the study before the screening period; 2) 3 days to 1 day prior to administration; 3) when one of the subjects who had received the gene therapy had to have an amputation after worsening of a toe ulcer; 4) when a serious complication of myelodysplastic syndrome occurred in one subject after 20 months of administration; and 5) when interstitial pneumonia occurred in a subject after 25 months of administering the gene. These timings and the decision to obtain informed consents vary according to the characteristics of the gene product and vectors, but the SeV vector affects the inflammatory system; therefore the primary investigator decided on the timings of the updates. The explanatory sheet and consent forms should be approved by the IRB and changes in content amended as an edition before use in patients.

Adequate patient education is crucial with gene therapy. The research nurse needs to reinforce information regarding gene therapy as well as safety instructions for patients and their families. To educate patients and their families, the research nurse is required to have a deep understanding of gene therapy, including basic concepts involving the molecular background of the disease, molecular genetics, immunology, gene technology, and results of preclinical studies. Anderson (2008) conducted a survey in 55 gene therapy study nurses, and stated that 25.5% had been prepared for their role as gene therapy study nurses by mentoring from the principal investigator, and 18.2% were prepared by self-teaching from additional reading and attending conferences. Because there are few educational programs tailored to suit research nurses involved in gene therapy, many still learn about gene therapy on the job and by being taught by physicians [36], a similar finding to that reported in 2001 by Mueller. As treatment progresses, new questions will be posed by patients and their families, and research nurses need formal on-the-job orientation programs and training continuously through the gene therapy. In the case of the first-in-man gene therapy trial using SeV, the principal investigator and the principal researcher educated all interdisciplinary team members and therapy-related department sections in the hospital, such as the nursing department, ward staff, laboratory staff, cell processing center staff, and also physicians. These education sessions were reported in formal forms as required by the Ministry of Health, Labour and Welfare in Japan as a part of the GCP act.

Furthermore, research nurses should be aware of the financial and insurance issues regarding gene therapy to inform the patients and their families [37]. Gene therapy is still an experimental therapy that patients can receive as part of a research study. Therefore, the financial obligations of such studies, including gene transduction, procedures, gene products, and tests related to the research are generally provided free of charge. In applying for permission from the Ministry of Health, Labour and Welfare of Japan to conduct gene therapy using SeV, the gene therapy researchers are required to take out a research insurance policy to cover unexpected medical damages as compensation for the patients. The requirement for a research insurance policy is applied to all clinical trials in Japan, and specifies that patients and their families must be informed before giving consent.

Safety

The safety of gene therapy is an important issue that participants and medical staff must consider [38]. One of the major concerns in gene therapy is the possibility of infectious transmission of recombinant genes with viral vectors [39]. Shedding of the virus and a risk of virus leakage resulting in systemic exposure can be determined by measuring the levels of genome copies using nested real-time reverse transcription-polymerase chain reaction as well as hemagglutinating activity, and requires checking at baseline and after treatment. The infectious spread of viruses has not been observed in gene therapy, but it is not clear what level of precautions are required. Therefore, universal precautions are usually applied when taking care of gene therapy patients because they are known to be effective in preventing the transmission of common pathogens such as hepatitis and human immunodeficiency viruses [39,40]. In most cases, gene therapy is administered in negative-pressure rooms, and specimens are treated and tested in level 2 biohazard laboratories. Clinical waste is required to be autoclaved.

The Cartagena Protocol, which is an act on the conservation and sustainable use of biological diversity through regulations on the use

of modified living organisms (Act No. 97, 2003), is strictly applied to gene therapy in Japan. The facilities that are scheduled to perform gene therapy are inspected in accordance with the Cartagena Protocol, and require approval from the Ministry of Health, Labour and Welfare. The inspections include the negative-pressure treatment rooms, the cell proceeding center, and the level 2 biohazard laboratories. The isolation period depends on the regulations in each country performing gene therapy and the characteristics of the vectors. In our case of using SeV first in humans, subjects were isolated and observed in the gene therapy rooms up to 7 days after administration. On day 7, after confirming the virus shedding results were negative, dressings were removed and subjects transferred to the general units. The genome was no longer present after 15 days in the first-in-man clinical trial using SeV [34]. After safety was confirmed in the trial, the isolation period was shortened by 1 day in the next phase of the trial.

An 8-year follow-up of gene therapy using VEGF gene transfer for coronary artery disease patients reported that gene therapy was relatively safe, with no associated risk of cancer or other diseases [41]. Long-term follow-up will be needed to establish the safety and efficacy of gene therapy.

Other Nursing Implications

As in other clinical trials, research nurses need to provide leadership to coordinate not only the medical procedures, but also the multidisciplinary medical team. Research nurses are often described as a vital link between the patient, principal investigator, study sponsor, laboratory staff, ward nurses, and administrative staff [42]. In addition, taking part in a gene therapy clinical trial is often a stressful experience for patients and their families. Therefore, psychological support is essential. Research nurses play the role of patient advocate to ensure the privacy and confidentiality of the treatment, and to ensure the appropriate conduct of the gene therapy trial.

A vital competency for the research nurse is skill in observing unknown symptoms. Because gene therapy and the use of viral vectors are innovative therapy, there are no specified predictable symptoms, prognosis, and synergic effects of therapy. Using a viral vector predicts inflammatory symptoms, but angiogenic growth factors affecting the gene transfer system may cause unknown effects. The research nurse provides an essential role in monitoring and assessing patients. The ICH guideline for Good Clinical Practice clearly describes that the aim of monitoring and assessing patients is to ensure the safety and rights of the trial subjects and quality of the data. There are no standard nursing guidelines for gene therapy nor for care of patients receiving viral vectors. Research nurses are required to assess unknown symptoms with the utmost care and attention and to provide quality nursing care for patients.

Conclusion

As scientists have progressed gene therapy research, nurses have learnt valuable lessons about caring for patients undergoing treatment. Long-term follow-up will be continuously needed for both patients and their offspring to establish the safety and efficacy of gene therapy. Gene therapy is developing steadily and rapidly. Overcoming its challenges has been essential in examining the complex and cumulative effects that key intersections between legal, social welfare, and labor market systems may have on the health of patients with no other option. Nurses have faced the challenge of supporting gene therapy, but the work has been rewarding, with many lessons learnt.

Gene therapy nursing continues to evolve within a multidisciplinary research team and contributes to the advancement of nursing science.

Conflicts of Interest

Dr Yonemitsu was a previous member of scientific advisory board of DNAVEC Corporation, which has developed SeV/dF-hFGF2. The other authors declare that they have no conflicts of interest. The all authors declared no potential conflicts with respect to the research, authorship, and/or publication of this article.

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