



REVIEW ON APPLICATION AND PRINCIPLE OF GENE THERAPY IN VETERINARY MEDICINE

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ABSTRACT

Gene therapy is a new modality with the potential for treating or preventing a variety of inherited or acquired diseases. For this purpose, there are a wide variety of gene delivery methods including viral and non-viral vectors. Commonly used viral methods included:retrovirus; adenovirus and lentiviral systems are suitable for gene therapeutic approaches which are based on permanent expression of the therapeutic gene.Non-viral methods included physical forces and chemical compounds to transfer DNA into a cell.Non-viral vectors are far less efficient than viral vectors, but they have advantages due to their low immunogenicity and their large capacity for therapeutic DNA. To improve the function of non-viral vectors, the addition of viral functions such as receptor mediated uptake and nuclear translocation of DNA may finally lead to the development of an artificial virus.The current literature review sets out many different models that are currently being investigated to bridge from studies in cell lines through towards clinical reality. Animal models include horse, dog,sheep, and Non-human primate models (macaque, common marmoset, owl monkey) are used for preclinical gene vector safety and efficacy trials to bridge the gap prior to clinical studies for the safety development of clinically effective delivery systems of DNA and RNA technologies. The major challenges have been delivery of DNA to the target cells and duration of expression.Ethical and moral issues implicit in gene therapy have drawn notice from several governmental and religious organizations.

KEYWORDS : Gene therapy, Delivery systems, Non-viral vector, Viral vectors,Animal Models**Introduction**

Gene therapy is a therapeutic technique in which a functioning gene is inserted into a cell in order to ameliorate a metabolic abnormality or to introduce a new function is one of the outcomes of breakthroughs in molecular biology (Patil et al., 2012). Gene therapy is a new modality with the potential for treating or preventing a variety of inherited or acquired diseases (Soboka et al., 2016) and is a promising approach to the treatment of cancer and other genetic diseases in human and veterinary medicine (KO and Abatan, 2008).

The first gene therapy trial in humans was conducted at the beginning of the 1970s and it was observed that naturally occurring DNA and RNA tumor viruses successfully delivered new genetic information in genomics of mammal cells (Escors and Breckpot, 2010). Due to developments in the science of genetics, at the beginning of the twentieth century, it was understood that diseases such as hemophilia were genetic diseases. Similarly, it was found that diseases such as colon cancer, diabetes and retinoblastoma were also genetic based diseases.

In 1980s, gene transfer to mammalian cells came forth after the development of retroviral vectors and became a routine procedure. Retrovirus-based gene therapies brought significant advantages as they can stably integrate their genomes to host-cell chromosomes. Since the late 1980s, DNA came to the stage as a genetic material. Later, it structurally got analyzed and further advances allowed the modification of genetic code. These discoveries on genetic material made cloning possible (Escors and Breckpot, 2010).

Progresses in vector targeted gene therapy, improves both gene therapy safety and fulfils its potentials as a therapeutic modality. Continued progress has been made in the development of viral systems including retrovirus, Adenovirus, Adeno associated, herpes virus as well as the exploitation of novel tools such as plasmid DNA herpes virus based systems (Soboka et al., 2016).

Diseases treatable by gene therapy were categorized as either genetic or acquired. Genetic diseases are those typically caused by the mutation or deletion of a single gene. Conversely, a single gene is not defined as the sole cause of acquired diseases. Though gene therapy was initially used to treat genetic disorders only, it is now used to treat a wide range of diseases such as cancer, peripheral vascular diseases, arthritis and neurodegenerative disorders (Mhashilkar et al., 2001). The expression of a single gene, directly delivered to cells by a gene

delivery system can potentially eliminate a disease. Prior to gene therapy studies, there was no alternative treatment for genetic disorders. Today, it is possible to correct genetic mutation with gene therapy studies (Sullivan, 2003). However, those gene therapy focused are studies independently available in fragmented fashion. Hence, the objectives of thereview were to organize and compile gene therapy studies in comprehensive handbook, overview animal models, revise gene delivery mechanisms and indicate challenges and ethical issues.

Gene Delivery Methods

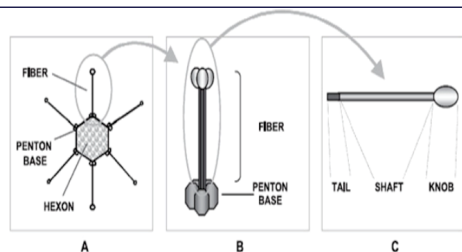
Broadly speaking, there are two main ways of transferring genes to the target cells.

Viral gene delivery systems

Since a large number of viruses have appropriate mechanisms for transfer of genetic material to the target cell, current gene technologies concentrated on the use of viral vectors that provide high transduction effectiveness and advanced level of gene expression. The optimal design of a viral vector depends on the types of virus to be used such as Adenovirus, Retrovirus, Lentivirus etc (Wunderbaldinger et al., 2000).

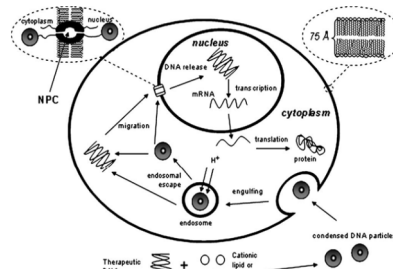
Adenoviral systems are one of the mostly used techniques 'of viral gene delivery systems for delivery system of gene therapy(Campos and Barry, 2007). Adenoviruses are commonly used as gene vectors (Dinh et al., 2005). C group adenoviruses Ad2 and Ad5 are the most widely studied adenoviruses. The capsid of an adenovirus determines virus tropism. Groups A and C–F first bind to highly-expressed coxsackie virus B-adenovirus receptor and thus realize their high infectivity in many tissues. In contrast, group B binds to complement-regulatory protein CD46. Adenoviruses replicate withinthe nucleus of the infected cell and produce virions (Dinh et al., 2005). These vectors have the ability to replicateand purification of the vectors generally involves easier and shorter processes (Armendariz-Borunda et al., 2011). Moreover, adenoviruses have important characteristics which make them indispensable for genetransfer. The most important ones are their well-known molecular biology, delivery capacityof foreign DNA fragments up to 36kb and ability to transfect DNA into many cell types(Sullivan, 2003).

Figure 1: Schematic of the adenovirus capsid (A) Whole capsid identifying fiber, penton base and hexon. (B) Enlargement of circled region in (A), showing homotrimeric fiber bound to homopentameric penton base. (C) Fiber monomer, identifying tail, shaft, and knob domains (Medina-Kauwe, 2003).



underlying gene therapy non-viral gene delivery system is that it develops gene expression to specific cells in order to treat human diseases or for transfer of genetic material to inhibit the production of a target protein using physical methods and chemical methods (He et al., 2010). A successful gene delivery procedure involves minimizing potential inhibitory inflammatory response while overcoming certain barriers at each step of the gene delivery procedure in order to optimize gene activity (Conwell and Huang, 2005).

Figure 2: Non-viral gene therapy method (He et al., 2010).



Physical gene delivery systems

Physical delivery systems create transient membrane pores for facilitating the gene transfer from extracellular to nucleus using physical forces. These methods include light, electric or magnetic field, electric pulse, particle impact, ultrasound, hydrodynamic pressure, local or rapid systemic injection, laser irradiation, microinjection, Naked DNA, electroporation, Sonoporation, magnetofection and others (Jafari et al., 2012).

In microinjection, cell membrane or nuclear membrane is penetrated by simple mechanical force using a needle of 0.5 μm -5 μm diameters (Manjila et al., 2013). This gene delivery system is mainly used to inject DNA constructs in vivo (Gascón et al., 2013). Naked DNA alone is able to be transferred into skin, thymus, cardiac muscle, and especially skeletal muscle and liver cells when directly injected, also it has been applied directly. Though naked DNA injection is a safe and simple method, its efficiency for gene delivery is low in comparison to other methods (Varga et al., 2001).

Electroporation uses electrical pulse to generate transient pores in the plasma membrane allowing efficient transfer of DNA into cells (Khan, 2010). This approach has been effectively applied in humans in order to enhance gene transfer and tested in several clinical trials such as leukemia, brain carcinomas, prostate cancer, colorectal cancer, malignant melanoma, Alzheimer, Parkinson, and depression (Amer, 2014). The major disadvantage associated with this method is that it often results in a high incidence of cell death (Nouri et al., 2012). Ultrasonic frequencies are used to make nanometric pores in membrane to facilitate intracellular delivery of DNA particles into cells of internal organs or tumors so the size and concentration of plasmid DNA have great role in efficiency of the system. The most important limitation of the system is low efficiency (Jianget al., 2001). The magnetic fields are used to concentrate particles containing nucleic acid into the target cells. Magnetofection is a simple and efficient transfection method that has the advantages of the non-viral biochemical (cationic lipids or polymers) and physical (electroporation, gene gun) transfection systems in one system while excluding their inconveniences such as low efficiency and toxicity (Yang et al., 2001).

Chemical gene delivery systems

Chemical methods are other non-viral methods that have been known as an important delivery system designed as natural or synthetic compounds such as polymers, lipids, peptides, and inorganic methods. Most polymers applied for gene therapy contain positive charge groups (amines) which interact with the negative charge groups of DNA (phosphates) to form compact structures named as polyplexes. These structures can be endocytosed by cells similar to lipoplexes (Bolhassani et al., 2014). Some inorganic materials such as gold, silica, calcium phosphate or magnetic nanoparticles can bind to the plasmid DNA and deliver it through endocytosis into the cells (Wegman et al., 2013). Transfection efficiency is very moderate for these methods though they have several advantages, such as low toxicity, good shape control and easy storage ability for further concentration on improving these types of delivery agents (Wegman et al., 2013). Many types of cationic peptides are able to interact with plasmid DNA as a safe option for gene therapy. Moreover, studies showed that the linkage of a

Retroviral systems are other widely used techniques for delivery system of gene therapy. The ability of retrovirus-based gene delivery vectors to carry foreign genetic material was first realized in the early 1980s (Escors and Bretpot, 2010). Retroviruses are diploid, single-stranded, circular-enveloped RNA viruses of the family Retroviridae (Escors and Bretpot, 2010). They cause diseases such as leukemia and cancer; however, their use as a vector in gene therapy brought new developments in treatment (Pages and Danos, 2003). Retroviruses are viruses that integrate with host genome to produce viral proteins which are extracted during gene delivery. Retroviral vectors have the capacity to deliver DNA up to 8 kb (Navarro et al., 2008). An ideal retroviral vector for gene delivery possesses cell-specific, regulated and safe properties. Effectiveness of delivery is important as it will also determine the effectiveness of therapy (Hu and Pathak, 2000). A retrovirus infects the target cell by providing interaction between viral envelope protein and cell surface receptor on the target cell. The virus then is internalized by the cell and its single-stranded RNA turns into double-stranded DNA (Yi et al., 2011). Double-stranded DNA is delivered to the nucleus and integrated to the host cell genome there. The arrival of a viral genome within the nucleus stabilizes the binding of viral DNA to the host genome providing an advantage of long-term expression of transgenes required for therapeutic effect. However, one of the disadvantages of current retroviral transfer systems is that they are not specific to types of target cells (Yi et al., 2011) but importantly they can integrate a reverse transcribed genome to the host cell chromosome (Miyazaki et al., 2011).

Lentiviral systems are other viral gene delivery systems for gene therapy which share common characteristics and similar structures with retrovirus as they stem from the same taxonomic family, Retroviridae (Wilson, 2013). Lentiviruses are enveloped and spherical and possess 2 copies of a single-stranded RNA genome measuring approximately 80 to 100 nanometers in diameter. Some lentiviruses are human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV). These viruses are known for having gene integration properties that make them highly suitable for use in gene therapy (Nisole and Saib, 2004).

The RNA genome from a lentiviral vector is altered to contain a therapeutic gene. When the vector infects a target cell, the therapeutic gene is reverse transcribed to DNA in the target cell's cytoplasm by a reverse transcriptase enzyme carried by the vector. Once transcribed, the DNA with the therapeutic gene enters the nucleus of the cell where it integrates into the genome of the target cell (Nisole and Saib, 2004), meaning the viral vector copies its genetic material by inserting the material into the target cell's DNA. Lentiviral vectors are highly successful in crossing the nuclear membrane of the target cell and permanently changing the cell. This advantage potentially increases the efficacy and longevity of therapeutic treatment. When therapeutic genes are integrated into a target cell's genome, new cells are created through mitosis. In mitosis, or nuclear division, the daughter cells are genetically identical to the parent cell. Daughter cells from a target cell infected by a lentiviral vector also contain the therapeutic gene which allows for the stable and long-term expression of the therapeutic gene (Miller, 2014). The difference between lentiviral and retroviral vectors is that lentiviruses can infect both quiescent (nondividing) and mitotically active cells, whereas retroviruses can infect target cells only during division (Cooray et al., 2012).

Non-viral gene delivery systems

Cell targeting refers to delivery of the therapeutic agent to a specific compartment or organelle of the cell. This is the most commonly used mechanism in endocytosis gene therapy, particularly in cellular uptake of non-viral gene delivery systems (Prokop and Davidson, 2007). After the cellular uptake of the delivery system by endocytosis, cellular release takes place to initiate DNA transcription and translation and to produce the related protein. The basic concept

peptide to a lipoplex or polyplex allows targeting to specific cell types (Gascón et al., 2013).

Animal models in gene therapy

Gene therapy like any other administration of a new drug has to be tested in animal models before clinical application in humans. The early animal models for gene therapy were focused upon simulated inherited (genetic) disorders, judged to be appropriate candidates for early human trials and based heavily upon molecular medicine in animals genetically engineered to reflect the disease state (Singh, and Johnson, 2006). Transgenic animals are used to model different human diseases: infection, neurodegeneration, apoptosis, arteriosclerosis, ageing, cancer, xenografts, etc. (Singh, and Johnson, 2006). One goal is to use these models to assess the potential efficacy of a genetic intervention by evaluating its impact on meaningful surrogate or clinical endpoints. Clearly, most of the gene transfer experiments have been performed in mice. Large animal models, such as pigs or horses, are almost exclusively used to study treatment of induced diseases, cardiovascular disease and arthritis, respectively (Casal, and Haskins, 2006). Sheep are used for the development of gene transfer techniques, gene marking studies, and assessment of safety, but despite the existence of genetic diseases in these species, they have not yet been used for treatment trials. However, aspects of the human population that are impossible to simulate in animal models are the tremendous environmental and genetic diversity. This will be particularly important in the context of immune responses to gene replacement therapy where several factors may profoundly contribute to outcome, such as nature of mutations in the disease gene, major histocompatibility genotype, and previous exposure of the recipient to the delivery vehicle in the context of naturally acquired infection (Galletti et al., 2007). These cell line studies typify a lot of current pharmaceutical research in non-viral gene therapy. Our own work on DNA condensation and cell-line transfection is similarly poised to make the transition in to animal models for target diseases in gene therapy (Adjimatera et al., 2008).

Equine models

The horse is a good model for osteoarthritis as the disease occurs naturally in this species. Osteoarthritis (OA) in horses and in humans is a significant social and economic problem (Frisbie and McIlwraith, 2000). Adenoviral-mediated gene transfer was used to investigate the therapeutic effects resulting from intra-articular overexpression of the equine interleukin-1 receptor antagonist gene in an established model of equine osteoarthritis that mimics clinical osteoarthritis. In vivo delivery of the equine IL-1RA gene led to elevated intra-articular expression of interleukin-1 receptor antagonist for approximately 28 days, resulting in significant improvement in clinical parameters of pain and disease activity, preservation of articular cartilage, and beneficial effects on the histological parameters of synovial membrane and articular cartilage (Frisbie et al., 2002). In horse, an osteochondral fragment was created in one randomly selected intercarpal joint, to produce an experimental OA, and the opposite joint served as the control. Fourteen days after surgery, they received Ad-EqIL-1RA viral particles/joint diluted with Gey's balanced salt solution (GBSS) in their joint with a lesion, while the opposite nonfragmented joint received a similar volume of GBSS. Clinical examination of the horses showed that the therapeutic expression of IL-1RA significantly decreased signs of joint pain as measured by degree of lameness (Frisbie et al., 2002).

Sheep models

Lehn and co-workers reported transfection of foetal sheep airways in utero using guanidinium-cholesterol cationic lipids (Luton et al., 2004). Gene therapy, to reduce rejection-mediated damage, holds out some promise as a novel therapeutic strategy in corneal transplantation as the donor cornea can easily be manipulated, ex vivo, prior to transplantation (Klebe et al., 2001). The corneal endothelium is the major target in human corneal graft rejection. As these cells are essentially postmitotic, any such damage cannot be repaired through cell division. The sheep is a useful model in this respect, as ovine endothelial cells are amitotic. In the absence of topical immunosuppression, corneal allografts become spontaneously vascularized and undergo irreversible rejection at 3 weeks postoperatively in a manner that is clinically and histologically similar to human corneal graft rejection and therefore particularly useful (Klebe et al., 2001).

Canine models

Over 50% of genetic diseases present in the dog are true orthologues of

human diseases caused by mutation in the same genes. In addition to the obvious longevity and similarity in size to a small child, many parts of the canine immune system are similar to those of the human (Sleeper et al., 2004). Mucopolysaccharidosis VII (MPS VII, Sly syndrome, β -glucuronidase deficiency) is an inherited lysosomal storage disorder caused by deficiency of β -glucuronidase activity, required for the catabolism of glycosaminoglycans whose accumulation in CV cells leads to cardiac disorders and also to CNS diseases. The cDNA sequences are known and the mutation has been identified. Some experiments tested intravenous retroviral (RV) vectors in neonatal dogs at days 2–3 of life (Xuet et al., 2002) improving the growth of treated dogs, and the skeletal disease was treated in their limbs (86). Hemophilia A and B have also been studied in dogs. They are x-linked inherited bleeding disorders caused by a deficiency of the blood clotting factor in response to bleedings crises. The canine models of haemophilia are useful for developing and evaluating gene therapies because the canine proteins are very well characterized, the genes have been cloned, and cDNAs are available (McCormack et al., 2006).

Challenges of gene therapy

Gene therapy researchers must answer several questions to gain approval for gene therapy trials in humans. They must determine whether the disease being treated is a good candidate for gene therapy and be certain that the gene they introduce will be correctly inserted and regulated so that it is clinically expressed in the patient. Researchers also must explain technical details of the DNA and the vector they will use. Even if these questions are answered, human gene therapy experiments can be delayed because of the technical aspects involved, risks to study participants and future patients, and the fear of human genetic engineering (Bergeson, 2014). During the 1980s, when gene therapy was in its infancy, representatives of government agencies and government-appointed groups debated the topic extensively, resulting in a succession of guidelines that legitimized gene therapy clinical trials. The emergence of a scientific discipline of gene therapy in the 1990s stimulated mixed views within and outside of the scientific community, episodes of public excitement and some ill-conceived and unsuccessful clinical trials (Wilson et al., 2013).

Ethical concerns of gene therapy Gene therapy manipulates cells in the human body. Therefore, its application is accompanied by several unique ethical and moral concerns. Inevitably, issues regarding affordability and economic fairness will arise (Hudson and Orviska, 2011). Ethical and moral issues implicit in gene therapy have drawn notice from several governmental and religious organizations. Debate regarding use of genetically engineered material in human subjects has been complex, with viewpoints from the fields of law, medicine, politics, biology, philosophy, and religion (Ormandy et al., 2011). Therefore ethical concerns, including animal welfare issues, can emerge at various phases in the propagation and life span of a respective genetically engineered animal (Ormandy et al., 2011). An adequate ethics of animal use in science which include theory of the Three Rs (Reduction of animal population, Refinement of enforcements and farm managements to curtail affliction and despair, Replacement of animals with non-animal surrogate wherever necessary (Avey and Griffin, 2016) is in place in addition to the application of the principles of humane experimental approach (Ibrahim, 2006).

Conclusion and recommendations

Theoretically, gene therapy is the permanent solution for genetic diseases. The review indicated the existence of reports on gene therapy. Technically, a gene is inserted into the genome to replace an "abnormal" disease-causing gene. Viral and nonviral methods are available to transfer genes to target cells. Various animal models are available to investigate target diseases using new technologies of gene therapy. Gene therapy is beneficial but could also exert negative impacts depending on how it is applied. Based on the above conclusion, continuous advanced research is recommended.

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