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Contemporary Animal Models For Human Gene Therapy Applications

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Abstract

Over the past three decades, gene therapy has been making considerable progress as an alternative strategy in the treatment of many diseases. Since 2009, several studies have been reported in humans on the successful treatment of various diseases. Animal models mimicking human disease conditions are very essential at the preclinical stage before embarking on a clinical trial. In gene therapy, for instance, they are useful in the assessment of variables related to the use of viral vectors such as safety, efficacy, dosage and localization of transgene expression. However, choosing a suitable disease-specific model is of paramount importance for successful clinical translation. This review focuses on the animal models that are most commonly used in gene therapy studies, such as murine, canine, non-human primates, rabbits, porcine, and a more recently developed humanized mice. Though small and large animals both have their own pros and cons as disease-specific models, the choice is made largely based on the type and length of study performed. While small animals with a shorter life span could be well-suited for degenerative/aging studies, large animals with longer life span could suit longitudinal studies and also help with dosage adjustments to maximize therapeutic benefit. Recently, humanized mice or mouse-human chimaeras have gained interest in the study of human tissues or cells, thereby providing a more reliable understanding of therapeutic interventions. Thus, animal models are of great importance with regard to testing new vector technologies *in vivo* for assessing safety and efficacy prior to a gene therapy clinical trial.

Keywords

Animal models; Gene therapy; Genetic diseases; Viral vectors; Humanized mice; Clinical trials

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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

INTRODUCTION

Gene therapy refers to the replacement of a defective disease-causing gene with a normal functional copy of the same gene to treat genetic diseases. Genetic mutations result in either loss or gain of function of genes leading to several genetic disorders including cancer. Therapeutic genes are delivered by either non-viral (physical or chemical) or viral methods into specific target cells [1, 2]. Viral vectors are highly efficient in gene transfer both *in vitro* and *in vivo;* however, major concerns such as immune response and insertional mutagenesis still remain [3]. Animal models are valuable tools in biomedical research to assess variables related to the use of viral vectors such as safety, efficacy, dosage and localization of transgene expression before moving onto an eventual human trial. A facility accommodating large animals like dogs, monkeys, and pigs is a cost-and-infrastructure intensive set-up as compared to a facility housing small animals like mice, rats and rabbits. Small animal models are a lot easier to handle and maintain, and since they have a short life span, they could be used to assess safety and efficacy within a short duration unlike large animal models. Though mouse genome is 99% similar to that of humans, [4] they do differ to some extent in the immunological make-up. On the other hand, using a large animal model could help with dosage adjustments, for instance, therapeutic interventions in dogs could very well correlate with small children [5]. Moreover, supportive studies on large models could be beneficial based on primary experimental conclusions made from small models to further improve therapeutics. A comparison between small and large animal models is summarized (Table 1). Here, we highlight some of the animal models of human diseases that are widely used in many gene therapy studies.

MURINE MODELS

Laboratory mice and rats are a favorite as they can be handled and maintained with ease. The litter size is more per cycle, thereby providing larger number of experimental animals. Mice are usually inbred and backcrossed resulting in genetically identical siblings in which case findings cannot be correlated with humans carrying genetic variations and signature gene polymorphisms. In the past, researchers have used spontaneous mutation models such as the severe combined immunodeficiency (SCID) mice [6], which were used in cancer gene therapy studies to model different types of cancer due to their naturally immune compromised state [7, 8]. Limitations of spontaneous mutation models include i) the intermittent rate at which these mutations occur [9] and ii) timely breeding based on visual identification of phenotypic differences.

With the advent of gene manipulation techniques, it is now possible to genetically engineer monogenic disease models within a short period of 6 to 8 months using methods such as zinc-finger nucleases (ZFN) [10, 11], transcription activator-like effector nucleases (TALEN) [12, 13] and a more recently developed clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 [14, 15]. These techniques are also recently reviewed elsewhere [16, 17]. Multiple gene knockout mouse can also be generated with ease using multiple CRISPR/Cas9 vector constructs [18–22], which would be very useful to model complex genetic disorders involving multiple genes. A recent application of this new technology is generation of the Spata7 gene knockout mouse to model congenital eye

disorders such as Leber's congenital amaurosis (LCA) and juvenile retinitis pigmentosa (RP) [23, 24]. Genetically engineered mouse (GEM) models of metabolic diseases and cancers are of great interest in the study of loss of function of tumor suppressor genes or gain of function of proto-oncogenes. One recent example is the generation of *Ace2* knockout mouse to mimic colitis leading to cancer [25]. Older cancerous xenograft models and chemically induced cancer models have limitations such as immunocompromised state and physiological side effects where the animals fail to resemble the disease and hence are unable to accurately respond to treatment. Taneja *et al.* gives an insight on few crucial transgenic and knockout mouse models of tumor suppressor genes [26].

Murine models are used in various gene therapy studies including those for cancer [27, 28], muscular dystrophies [29, 30] hematological [31, 32], neurological [33], respiratory [34], liver [35] and cardiovascular disorders [36]. In general, it is always a good practice to evaluate a novel therapeutic strategy in an animal model first before performing the same on human subjects to help assess safety and improve therapeutics. Sometimes, mouse models do not entirely mirror phenotypic features of human diseases in which case it becomes difficult to interpret and correlate crucial findings for human applications [37, 38]. In one study, lentiviral vectors were customized to improve specificity and potency for the treatment of chronic granulomatous disease (CGD) by gene therapy using tissue-specific and gene-specific promoter, $gp91^{phox}$ to drive therapeutic gene expression in phagocytes. The most promising vector giving therapeutic levels of expression was used to treat CGD mice [39]. Following *in vitro* testing of newly designed vectors, potential ones could be picked to determine their efficacy *in vivo* using small preclinical models.

A comparative preclinical study between adult mice and non-human primates (NHPs) showed that intravascular administration of adeno-associated viral vector serotype 9 (AAV9) was able to transduce and actively cross the blood-brain barrier comparatively better in mice than NHPs where poor transduction into brain and peripheral organs was observed. This could be due to the presence of low levels of pre-existing neutralizing antibodies (NAbs) in NHPs that could have blocked transduction into the brain and peripheral organs [40]. These results conclude that the presence of pre-existing NAbs, peripheral tropism and limited neuronal transduction are major concerns impeding human translation using AAV9 by intravascular delivery [41]. Similar differences in AAV9 transduction profile between dogs and mice have been previously reported [42, 43]. NAbs against AAV serotypes vary with respect to titers between species and serotypes, therefore prior screening for appropriate models for gene transfer experiments is crucial before conducting preclinical gene therapy studies [44].

Gene therapy was able to restore vision and delay retinal degeneration in a knockout mouse model of RP ($CNGB1^{-/-}$) after therapeutic administration of recombinant AAVs (rAAVs) into the sub-retinal space of a two-week old $CNGB1^{-/-}$ mouse [45]. This study provided proof of concept that could be further tested in a large animal model (e.g. dog or non-human primate) before translating the findings to humans. AAV2-RPE65 gene therapy to treat LCA in humans has been a true success story in the last decade [46–51].

In an anti-aging study, aged mice were induced to overexpress the enzyme telomerase by gene therapy. Telomerase has been shown to slow down the cells' biological clock, thereby increasing the life span by 24% with a single treatment and without increasing the risk of cancer in the adult mice [52]. Another study showed reversal of loss of memory by gene therapy in mouse models during the initial stages of Alzheimer's disease [53]. Preclinical testing for degenerative studies could be very time-consuming in a large animal model, whereas mice with their shorter life span could facilitate beneficial findings over a relatively short period of time.

It has been reported in a few cases that rat models when compared to mice were better in mimicking bioelectric phenotypes similar to those seen in human CF patients [34]. In addition, due to their smaller size and ease of handing, rats serve as better alternatives to large animals, yet recapitulating muscular lesions seen in human patients with Duchenne muscular dystrophy (DMD) [30].

CANINE MODELS

Dogs are excellent preclinical models used in many gene therapy studies. Over 58% of genetic diseases seen in the dogs closely depict the phenotype of human diseases caused by mutations in orthologous genes. The size of the animal is similar to that of a small child in which case dosage adjustments could be easily done. Immunological and clinical phenotypes in dogs are similar to those in humans most of the time [54].

Before using viral vectors to treat human diseases by gene therapy, the safety and efficacy of these vectors need to be first determined with the help of a reliable experimental model. For retinal gene therapies, consecutive local administration of rAAV vectors may be required for long-term expression of therapeutic gene in humans as the time window for administering the vector is unclear, although some preclinical reports claim that sustained expression could be achieved with one-time administration [55]. Immunosuppression for systemic bone marrow gene therapy requires non-myeloablative busulfan treatment in canine models to facilitate transplantation of gene-corrected hematopoietic stem cells [56]. In general, in order to boost levels and sustain expression of a therapeutic gene, immunosuppression in combination either with infusion of gene-corrected cells or administration of therapeutic gene-encoding viral vectors is necessary with systemic delivery [57]. Local administrations into the eyes have an immuneprivilege as they are compartmentalized and immune responses are limited to the injection site [58, 59]. The goal is to design a treatment modality involving minimal or complete elimination of prior immunosuppression that is commonly done in clinical practice.

Treatment of canine leukocyte adhesion deficiency (CLAD) using foamy virus vectors demonstrated amelioration of symptoms in four out of five CLAD dogs infused with vector-transduced autologous $CD34^+$ hematopoietic stem cells (HSCs) following a non-myeloablative conditioning regime leading to complete reversal of the CLAD phenotype [60]. All treated dogs have been shown to sustain CD18 expression and essentially disease-free for more than 4 years post-treatment. There were no genotoxic effects reported and further the risk of integration near oncogenes was shown to be much lower compared to that

of gammaretroviral vectors [61]. Disease characteristics in CLAD-affected dogs are similar to those in humans [62]. Therefore, dogs are considered as suitable large animal models for hematopoietic stem cell gene therapy [5]. In a few recent gene therapy studies, dogs have also been used as models for haemophilia [63, 64], ocular disorders [65, 66] and cardiovascular diseases [67, 68].

X-linked retinitis pigmentosa in humans and dogs is caused by defects in the retinitis pigmentosa GTPase regulator (RPGR) gene and results in early severe and progressive vision loss. It is one of the most common inherited forms of retinal degeneration in man. The defect was shown to be corrected with sub-retinal injections of AAV2/5 vectors carrying the human RPGR gene under the control of human interphotoreceptor retinoid-binding protein (IRBP) or G protein-coupled receptor kinase 1 (GRK1) promoters [69]. The similarities between humans and dogs in terms of eye anatomy, vision assessment [70–73], disease characteristics and positive responses to gene therapy provide hope towards human translation in the near future.

Diabetes is caused due to prolonged impairment of glycemic control. A gene therapy study was conducted involving two genes, glucokinase and insulin that work synchronously and detect high blood sugar levels promoting the uptake of blood glucose into target cells. Long-term efficacy of this approach in a canine model of diabetes was also demonstrated. A one-time intramuscular administration of AAV1 encoding *Gck* and *Ins* was used to treat insulin deficient diabetic dogs. This study provided the first proof of concept in a large animal model for a gene transfer approach to treat diabetes in humans [74]. A recent AAV8-mediated gene therapy in a dog model to treat myotubular myopathy with a single-dose intravascular delivery significantly improved severe muscle weakness and respiratory impairment, and extended the life span to more than 1 year with no toxicity or immune response [75].

NON-HUMAN PRIMATE MODELS

Non-human primates (NHPs) such as African green monkeys, baboons, chimpanzees, cynomolgous monkeys, rhesus monkeys and owl monkeys are considered the most suited animal models for preclinical testing as they are evolutionarily and genetically very closely related to humans compared to any other mammals. Rhesus macaques, in particular, have contributed immensely to several preclinical studies, although selection of a suitable model remains crucial considering differences in immunological responses to viral vectors between NHPs and humans which could impede translation into human gene therapy trials [40, 76–79]. Recently, successful generation of site-specific multiple gene targeted cynomolgus monkeys was demonstrated using the CRISPR/Cas9 system without any off-target effects paving way for generation of more genome engineered large animal models in the future [80].

NHP models accurately mirror the effectiveness of rAAV-mediated gene delivery to the airways in humans. Using an identical model system such as differentiated airway epithelial cells from the Indian Rhesus monkeys and from humans cultured at an air-liquid interface, the biology of rAAV-mediated gene transfer for three serotypes was assessed between the

two species. While the airway epithelia from NHPs and humans have similar Na^+ and Cl^- transport properties, rAAV transduction of airway epithelia of NHPs showed significant differences compared to those of humans in terms of the efficiency of transduction with all the three rAAV serotypes tested (AAV1, AAV2, AAV5) [81].

Another pilot study for cystic fibrosis (CF) gene therapy using a marmoset model showed that an intratracheal bolus dose of vesicular stomatitis virus G protein (VSV-G) pseudotyped HIV-1 based lentiviral (LV) vector carrying the *LacZ* reporter gene after airway treatment resulted in *LacZ* gene expression (X-gal). This was found primarily in the airway epithelia and alveolar regions. The *LacZ* gene expression was not seen in the liver or spleen. This study confirmed the transduction potential of CF-relevant airway cell types. The marmoset is an encouraging NHP model for testing and translating genetic treatments for CF ahead of clinical trials [82]. Marmosets are small and similar in body size to children and hence small volumes of viral vectors are sufficient for treatment which could be easily produced in the laboratory using the currently available methods of vector production.

NHPs have a vertebrate brain like that of humans in terms of neural circuitry, physiological and behavioral characteristics, which makes them a crucial and precise model for neurological diseases compared to other animals. Spontaneous atrophy of neurons occurs with aging in the brain of non-human primates similar to humans. Long-term expression of nerve growth factor (NGF) by LV-mediated intraparenchymal delivery has been shown to reverse the age-related cholinergic degeneration owing to sustained gene expression for one year in young monkeys [83]. These findings could pave way for potential testing in patients suffering from Alzheimer's disease. Another study reported to be effective and first of its kind is the AAV2 delivery to the NHP hippocampus using frameless stereotactic methods [84], which is very similar to the stereotactic delivery methods used previously in clinical gene therapy trials [85].

Red-green colour blindness, which results from the absence of wavelength sensitive visual photopigments is a common single gene eye disorder. Gene therapy was performed on adult squirrel monkeys that were congenitally colour blind due to the absence of L-opsin. Addition of a third opsin to correct the defect in vision resulted in trichromatic colour vision in the treated monkeys [86]. This study provided a positive outlook on treating congenital vision defects in humans.

HUMANIZED MOUSE MODELS

Mouse-human chimeras or "humanized mice" are immunodeficient mice designed to express human transgenes or engrafted with human cells or tissue [87]. The use of humanized mice is comparatively beneficial as they are designed to mimic human biology and features of human disease conditions, the treatment response in which could provide a precise model to address various health problems such as the immunodeficiency disorders. Although bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT) are currently available to treat various immune and genetic disorders, there are considerable limitations with each of these approaches [88]. Using some of the common immunosuppressed murine models such as the NOD/SCID (non-obese diabetic/severe

combined immunodeficiency) and NSG (NOD/SCIDγc$^{-/-}$) mice, human HSCs or patient-derived xenografts could be established for more precise clinical experimentation [89]. Due to differences in human and animal physiology, very often findings in any of the small preclinical models may not completely translate to humans. Hence, an *in vivo* model that closely mimics humans is better in order to translate findings from the laboratory bench to the clinical bedside.

Neutrophils in mice are different from that in humans in terms of their role, structure of surface molecules and certain signaling pathways. Recent research has made it possible to design immunodeficient mouse strains that would allow better engraftment of human hematopoietic cells by deleting the interleukin-2 (IL-2) receptor. Studies have demonstrated that functional human neutrophils tend to develop from human CD34^{+} cord blood stem cells in NOD-scid-γc−/− mice [90]. HIV-AIDS model and hu-HSC-transplanted NOD-scid-γc−/− mice have both been used in the treatment of HIV in various gene therapy studies [91]. Humanized models such as these could be used in the study of various human-specific autoimmune, metabolic, cancer and infectious diseases [92–96].

Similar studies could be carried out for leukocyte adhesion deficiency type 1 (LAD1). Using a mouse model, it has been shown that the therapeutic human CD18 subunit was able to heterodimerize with the mouse CD11a subunit [97]. On the other hand, if a humanized mouse engrafted with human HSCs were to be used, heterodimerization of the human CD18 would occur with all of the four human CD11 subunits such as CD11a, CD11b, CD11c, CD11d, as it would in a human system. Humanized mice could serve as strong preclinical models to understand the blood biology in health and disease and therefore have an important role in biomedical research in the future.

Humanized murine liver models such as the immunosuppressed FRG mouse (*Fah*$^{-/-}$*/Rag 2*$^{-/-}$*/Il2rg*$^{-/-}$) partially repopulated with human hepatocytes [98] were used to evaluate the efficiency of transduction of AAV variants into human hepatocyte xenografts. Out of 15 vectors tested *in vitro* and a few *in vivo* for various parameters such as the presence of neutralizing antibodies against the vectors [40], it was shown that the rAAV-LK03 serotype preferentially transduced human hepatocytes. This suggests that a human-mouse chimeric liver model could be used to accurately screen multiple gene therapy vectors for various factors such as virus entry based on serotype and species specificity and level of transgene expression post-infection, which could not have been precisely modeled in normal mice or NHP models otherwise [99].

PORCINE MODELS

Porcine models have served as desirable biomedical research models for a range of human diseases [100]. Preliminary data confirms the phylogenetic proximity of swine to humans as compared to the rodent species, which further closely relates them to humans in their physiology, histopathology, diet, metabolism and pharmacokinetics [101]. Many cases of porcine models with both spontaneous and targeted genetic mutations have been reported. The use of genetically engineered pigs is dramatically increasing in the field of biomedical disease modeling. This is due to their similarities to humans with regard to metabolism,

physiology, genome organization, aging and pathology. However, spontaneous mutant models have their own disadvantages in terms of risks related to insertion of transgenes into undesired locations. Transgenic models of pigs for many diseases like Alzeihmer's disease, diabetes, cancer, ophthalmological and cardiovascular disorders have been generated and found to be successful [102].

Swine is one of the most preferred models for cardiovascular diseases, as myocardial infarction could be produced in them in expected sizes and location with much ease. Also, its resting heart rates and left ventricular (LV) pressures are found to be analogous to humans. In one study, long-term expression of β-adrenergic receptor kinase carboxy terminus (β-ARKct) was evaluated to monitor cardiac function by AAV6-mediated myocardial gene delivery. β-ARKct is a peptide inhibitor of G protein-coupled receptor kinase 2 (GRK2), which is the key mechanism attributed to cardiac dysfunction. Therefore, AAV6 vectors encoding the β-ARKct gene was shown to result in long-term expression causing substantially significant amelioration of cardiac function and normalization of catecholaminergic axis and LV haemodynamics [103].

Several other studies stating the importance of porcine models for cardiac related disorders have been carried out where short-term over expression of inducible nitric oxide synthase (iNOS) gene was found to be effective in locally increasing nitric oxide (NO) levels thereby preventing intimal hyperplasia [104]. Another study showed that an alternatively spliced form of the vascular endothelial growth factor, $VEGF_{165}$ also correspondingly acts upon adult cardiomyocyte cell cycle, consequently increasing both mitotic index and nuclear hyperplasia eventually leading to cardiomyocyte regeneration in pigs [105]. The Na^{+}-Ca^{2+} exchanger/sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (NCX/SERCA2a) ratio targeting cardiomyocyte Ca^{2+} cycling required to reduce diastolic SR Ca^{2+} leak and increase systolic contractility of the cardiomyocyte is higher in large animal models like pigs and humans, thus forming a critical link in translating basic concepts into clinical therapies [106]. Another study showed that dual AAV trans-splicing and hybrid vectors could transduce pig photoreceptors, the major cell targets for the treatment of inherited retinal dystrophies (IRDs), to levels which were about two to threefold lower than those attained with a single AAV vector of normal size. This efficiency of dual AAV vectors is significantly higher than in mice, which is potentially due to higher levels of dual AAV co-transduction being observed in pigs [107].

Cystic fibrosis is one of the diseases where successful gene therapy could be a major boon. Ion transport defect i.e. lack of cAMP-stimulated anion transport in sinus epithelia were corrected in a porcine model using an adenoviral (AdV) vector. Porcine sinus epithelia were transduced using an AV vector expressing CFTR, which lead to restoration of cAMP-mediated anion transport indicating that CFTR expression by a comparatively small number of cells could restore anion transport, a successful step in disease correction which was first demonstrated in a porcine model of cystic fibrosis. However, this still leaves behind a question of alteration in CF phenotype in sinus hypoplasia through CFTR correction [108]. The long life span of swine makes it amenable to the study of a disease like CF where lung deterioration progresses over a comparatively long period of time paving way for potential novel therapeutics to be tested over time.

In haemophilia A, a bleeding disorder caused by abnormalities in the coagulation factor VIII, gene therapy enabled sustained elevation of factor VIII levels. Currently available mouse models failed to extrapolate the human condition as they could not show similar coagulation ability. Hence, a porcine model was generated from F8 targeted fibroblasts using nuclear transfer. The infusion of human factor VIII using F8 targeting vector, constructed by insertion of two genomic DNA into the plasmid vector pHSv-TK/PGK-Neo resulted in reduced bleeding [100]. This would make way for porcine models as efficient animal models for gene therapy of haemophilia A.

The use of a porcine model is not just limited to the above mentioned disorders but is also widely used for various muscular genetic disorders like DMD and spinal muscular atrophy (SMA). SMA is an autosomal recessive disorder resulting either from the loss or mutation of two independent genes, survival motor neuron 1 and 2 (SMN1 and SMN2), both of which code for the same protein, SMN [109, 110]. Most of the functional SMN protein is from the SMN1 gene, while a small part of the protein is produced from the SMN2 gene. Therefore, mutations and/or absence of both the genes could lead to a more severe phenotype. As SMN2 gene is seen only in humans, naturally occurring mutations in this gene are rare in other species. Like other animals, mice possess only SMN1, the loss of which is embryonically lethal [111]. Hence, SMA mouse model with SMN1 gene knockdown was developed which showed remarkable survival with the use of scAAV9-SMN vector encoding the normal functional copy of the SMN1 gene [112–114]. However, its relative permeability to blood-brain barrier and discrete symptoms like tail necrosis and cardiac defects that are unlikely seen in humans make the SMA mouse model unsuitable to study the human disease [115–117]. Hence, a large animal model like the porcine model, the very first for SMA, was developed by post-natal reduction of SMN protein in motor neurons for proper understanding of the disease phenomenon. A scAAV9-hSMN vector that was delivered at 4 weeks post-knockdown rescued the gene expression and also improved the neuropathology [118]. This indicates that the degeneration process in motor neurons due to low SMN levels could be reversed even after the appearance of symptoms. Thus, a large animal model like porcine could be beneficial in mimicking the human pathology, especially for gradual onset diseases.

RABBIT MODELS

Rabbits are more often considered for the production of monoclonal and polyclonal antibodies. However, they are increasingly being used as experimental models recently. *Oryctolagus cuniculus,* white New Zealand rabbit is extensively used for clinical studies. Transgenic rabbits have previously been chosen as animal models for diverse studies including lipoprotein metabolism, atherosclerosis, cardiovascular research and hypertrophic cardiomyopathy [119].

One study showed prevention of arterial thrombus formation i.e. promoting thromboresistance by local overexpression of tissue plasminogen activator in a rabbit model [120]. Tissue plasminogen activator (tPA) catalyzes the rate-limiting step, which involves the conversion of proenzyme plasminogen to plasmin in the presence of fibrin [121]. Recombinant adenovirus vector expressing human tPA under the control of Rous sarcoma

virus (RSV) promoter (Adv/RSV-tPA) was used to determine the impact of local tPA overexpression by delivering into the rabbit common femoral artery. Adv/RSV-tPA vector construct not only barred occluding intra-arterial thrombus formation, but also limited the complications by a decline in fibrin split product levels constantly seen with systemic haemorrhage [120]. Though transgenic mice are most extensively used for human diseases, it is found that in certain mutation cases they fail to display expected pathological symptoms. In terms of molecular composition of cardiac sarcomeric proteins, the mutant phenotypes were found to reflect more similarity with rabbits than rodents [119].

Another study where pathogenesis of transplant-related arteriosclerosis in rabbits due to smooth muscle cell replication was overcome by elimination of dividing cells for expression of herpes virus thymidine kinase (*hsv-tk*), which produces a toxic compound killing cells by phosphorylation of gancyclovir. Smooth muscle cells allow gene transfer only under de-endothelialization of arteries. Due to the resistance exhibited by these cells in gene transfer, certain manipulations to increase the porosity of arteries were made. These manipulations were successful in enhancing the efficiency of gene transfer in smooth muscle cells [122].

Limb ischemia is one of the prevailing problems due to lack of pharmacological interventions. The need for alternative treatments has led to the development of a novel strategy where angiogenic growth factors, such as hepatocyte growth factor (HGF) were shown to augment collateral artery development [123]. Intramuscular injection of HGF plasmid into rabbit ischemic hind limb not only showed significant augmentation of collateral vessel development, but also their extension from stem artery source to the end-point of reconstituted parent vessel. This study on rabbit ischemic hind limb models proved to be a potential therapy for peripheral arterial diseases thus making rabbits as efficient models for gene therapy.

For the very first time, a serious pathological condition like corneal neovascularization in the rabbit cornea has been significantly inhibited using decorin (proteoglycan). After examining multiple AAV serotypes (2, 5, 7, 8, and 9), AAV5, AAV8 and AAV9 were shown to lead to efficient transduction of the rabbit cornea. Superior transduction efficiency of AAV5 showed about 67% reduction in corneal neovascularization. Also, it was shown that decorin gene transfer into the cornea remarkably reduced VEGF induced corneal angiogenesis. Low inflammatory response with minimal confounding effects of wound healing enables a better understanding of molecular mechanisms with regard to corneal angiogenesis, eventually making rabbit the most frequently used animal model for angiogenesis research [124].

Safety of gene therapy approaches depends on various factors including vector dose, biodistribution and route of administration. Rabbits serve as excellent models to determine safety based on the above mentioned factors. In one such study, a rabbit model was used to characterize the safety of AAV serotypes i.e. AAV5 and AAV6 for liver-mediated human factor IX (hFIX) expression at different doses of 1×10^{12} or 1×10^{13} viral genomes/kg [125]. Both cohorts of AAV6 showed remarkable circulating therapeutic levels of hFIX, while slightly high dose of AAV5 was found to be effective. Also, no inhibitory antibodies to hFIX were found with AAV5 injected rabbits. While comparing the serotypes based on biodistribution, it was shown that AAV6 was found outside the liver and spleen in very low

concentrations, whereas AAV5 was found in many tissues and organs. Rabbit strains have shown varied genetic background compared to both inbred and outbred strains. This can be an advantage for studying intricate disease models in view of the fact that they mimic human genetic diversity with high accuracy.

OTHER ANIMAL MODELS

Feline Models

Cats are of great interest for neurological disorders [126] since their brain is >50 times larger than the mouse brain and also its anatomy is more similar to that of a human than a rat or a mouse [127, 128]. Sandhoff disease (SD) is a neurodegenerative lysosomal storage disease caused by catalytic deficiency of β-N-acetylhexosaminidase (Hexβ) responsible for step-wise catabolism of GM2-ganglioside by removal of its terminal N-acetylgalactosamine residue. Hexβ results in Sandhoff disease, whereas the defect in the α-subunit of Hex results in Tay-Sachs disease. Since optimal production of Hex α and β is required for co-expression of both the subunits [129–131], feline cDNAs of both the subunits were cloned in rhAAV8 vectors. Single intracranial injection of these vectors increased the life span of cats from 5 to 8 months. This increases the enzymatic activity to about 75-fold as compared to the normal with mild evidence of an immune infiltrate. Hence, feline model is preferred as an intermediate model between mouse and human studies for neurological disorders [132].

Bovine Models

The use of cattle for gene therapy is very rare, probably due to its size and also the high cost accrued due to large scale production of recombinant proteins and viral vectors [126]. However, using calves could be an alternative as they weigh around 30 kg, which is similar to that of an older child. Bovine model is the only available model for citrullinemia, an inborn error of urea cycle metabolism caused by deficiency of arginosuccinate synthatase leading to hyperammonemia. Inefficient pharmacological treatments where the oral administration of $^{15}N_4$ -labelled urea was used to measure the nitrogen flux led to the use of viral vectors. Systemic administration of a first-generation E1-deleted AdV vector expressing human arginosuccinate synthetase successfully resulted in the transduction of hepatocytes and partial correction of the enzyme defect leading to restoration of urea synthesis [133].

Equine Models

Horses are one of the most commonly used models for osteoarthritis as this condition occurs in them naturally [134]. Osteoarthritis is a chronic, debilitating and expensive disease involving the joints [135]. A recent study on the therapeutic effects resulting from the intra-articular overexpression of the equine interleukin 1 receptor antagonist (IL-1Ra) gene by AdV-mediated gene transfer was established. Elevated levels of intra-articular expression of IL-1Ra after the *in vivo* delivery of equine IL-1Ra gene were observed up to 28 days. This resulted in a significant improvement in various clinical parameters such as pain, disease activity and prevention of articular cartilage, also with some beneficial effects on histologic parameters of articular cartilage and synovial membrane [134]. Surprisingly, most of the

gene therapy experiments are performed in transgenic models instead of spontaneous mutant models such as the equine model.

CONCLUSION

Congenital and metabolic diseases caused by single gene defects leading to loss of function are viewed as potential candidates for gene therapy. Gene targeted knockout mouse model systems generated with the currently available gene manipulation techniques have their limitations as disease models for preclinical experiments which would make it difficult to develop novel therapies in an impending clinical trail. Therefore, it becomes a pertinent need to test large animal models like dogs and NHPs which are preferred based on various factors in close resemblance to humans such as similar size, anatomy, genetic background and disease pathology. Following an experimental trend from mice-to-dogs-to-humans could better validate various treatment modalities for diseases like cancer and other life-threatening human genetic disorders, thereby ensuring improved patient care in the future. Animals used to model human diseases affecting various organ systems are summarized (Table 2).

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Biography

E.J.R. Nelson

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Table 1.

A comparative look at small and large animal models.

Parameters	Small Models	Large Models
Handling and maintenance	Easy handling, inexpensive, minimal housing space, higher litter numbers	Laborious, expensive, larger housing space, fewer litter numbers
Phenotypic expression	Partially recapitulate complex human clinical features	Closely mirror clinical presentations of genetic disorders with respect to genes, anatomy and physiology
Disease modelling	Genetic disease models generated in less duration due to shorter gestation period (21 days) using techniques like ZNF, TALEN and CRISPR/Cas9	Takes longer duration to generate genetically modified animals due to longer gestation period (≥2 months)
Longitudinal studies	Restricted to shorter experimental studies < 1.5 years	Longer experimental studies of >2 years possible due to longer life span of the animal
Therapeutic interpretation	Dosage adjustments cannot be correlated for clinical applications	Dosage adjustments are similar to that of a small child

Table 2.

Animal models used in gene therapy for diseases related to various organ systems.

Animal Models	Organ Systems	References
Murine	Muscular system	[29, 30, 111, 114]
	Circulatory system	[31, 32, 36, 39]
	Cancer	[27, 28]
	Ocular system	[24, 45, 48]
	Nervous system	[33, 53]
	Aging	[52]
Canine	Endocrine system	[74]
	Circulatory system	[60, 61, 63, 64, 67, 68]
	Ocular system	[46, 47, 65, 66, 69]
	Muscular system	[42, 57, 75]
Non-human primates	Ocular system	[86]
	Nervous system	[83, 84]
	Respiratory system	[81, 82]
Humanized mice	Cancer	[95]
	Circulatory system	[88, 89, 92, 94]
	Immune system	[91, 96]
	Hepatobiliary system	[99]
Porcine	Circulatory system	[100, 103, 105]
	Respiratory system	[108]
	Ocular system	[107]
	Muscular system	[118]
Rabbit	Circulatory system	[120, 122]
	Ocular system	[124]
Other models (Feline, Bovine, Equine)	Nervous system	[132]
	Hepatobiliary system	[133]

Animal Models	Organ Systems	References
	Skeletal system	[134]