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Nonhuman primate model in clinical modeling of diseases for stem cell therapy

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Abstract:

Nonhuman primates (NHPs) are alike humans in size, behavior, physiology, biochemistry, and immunology. Given close similarities to humans, the NHP model offers exceptional opportunities to understand the biological mechanisms and translational applications with direct relevance to human conditions. Here, we evaluate the opportunities and limitations of NHPs as animal models for translational regenerative medicine. NHP models of human disease propose exceptional opportunities to advance stem cell-based therapy by addressing pertinent translational concerns related to this research. Nonetheless, the value of these primates must be carefully assessed, taking into account the expense of specialized equipment and requirement of highly specialized staff. Well-designed initial fundamental studies in small animal models are essential before translating research into NHP models and eventually into human trials. In addition, we suggest that applying a directed and collaborative approach, as seen in the evolution of stroke NHP models, will greatly benefit the translation of stem cell therapy in other NHP disease models.

Key words:

Myocardial infarction, nonhuman primate models, Parkinson's disease, stem cell therapy, STEPS, stroke, translational studies, Type-1 diabetes

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Introduction

Human and nonhuman primates (NHPs) have many similar attributes, such as behavior, physiology, anatomy, biochemistry, organ mechanisms, and immunology.[1]

Research suggests that NHP animal models possess the capability to link the translational research between small animal models and humans. NHP models of human disease propose exceptional opportunities to advance stem cell-based therapy by addressing pertinent translational concerns associated with this research. These translational aspects include the application of autologous/allogeneic-induced pluripotent stem cell (iPSC)-derived cellular products, concerns related to the immune response, delivery techniques in a clinical setting, and the evaluation of candidate cell line profiles when transplanted. NHP models offer unique opportunities to evaluate the complexity of the biochemical, physiological, behavioral, and imaging end points that are pertinent to current human conditions. Given the expense of specialized equipment and requirement of a highly specialized staff, the value of using these primates must be carefully assessed. Well-designed, less resource demanding, initial fundamental studies in small animal models, such as rodents, are essential before translating research into NHP models and ultimately into human trials. In this current report, we suggest that a robust dialog within each disease-specific research community focused on the development of relevant NHP models will greatly benefit the advance of the translation of stem cell research.

Nonhuman Primate Disease Models for Stem Cell-based Therapy

One of the most relevant NHP models is the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced NHP model of Parkinson's disease (PD). It provides significant preclinical opportunities that will ultimately demonstrate the safety and efficacy necessary to translate novel treatments into human trials. Like in humans,[2,3] MPTP-treated

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NHPs exhibit most difficult symptoms of sporadic PD, such as static tremor,[4] that can only be recognized in certain PD monkey models. In addition, MPTP injections in NHPs result in side effects to anti-parkinsonian drugs as experienced by humans with PD, such as dyskinesias with Levodopa Pharmacotherapy,[5] cumulate the major constituent, α-synuclein, of Lewy bodies, the pathological trademark of PD,[6] and exhibit cognitive disruptions.[7] NHP models of PD have already been instrumental to our understanding of the differences in autologous versus allogeneic PSC-based therapy, their ability to innervate the putamen, and the appropriate differentiation profile of dopaminergic neurons.[8-14]

Numerous research facilities have focused on various stem cell-based regenerative therapies for PD. This will yield important translational data to prove the potential of this therapy and recognize the possible clinical obstacles that must be addressed. As seen in Phase I studies, PD patients receiving neural grafts have shown an increase in their quality of life,[15,16] suggesting proof of the concept. However, the next hurdle to overcome remains the challenge of developing a predictable delivery system and sustainable stem cell populations that adhere to strict regulatory measures. Proven safety and efficacy of the therapy in NHP will effectively translate the product to commercial use and provide way to the development of a standardized treatment.

Type-1 diabetes mellitus (T1D) is an autoimmune disease that results in the permanent destruction of a specific cell type, the β-cells of the pancreatic islets of Langerhans; therefore, it represents an ideal candidate for cell replacement therapy. Indeed, islet transplantation started in 1894 with much improvement in the 1990s[17] with a landmark paper reporting the insulin therapy independence in seven out of seven T1D patients.[18] Clinical centers with experience in the transplantation procedure achieved 80% insulin independence in 80% of patients during the 1st year posttransplantation.[19] However, there was a high incidence of failure in long-term associated mainly with poor pancreatic graft and complications from permanent immunosuppression. Other limitations and challenges within the current approach are the reliance on organ donations, tissues processing for isolating quality-controlled islet cells, lifelong immunosuppression, and the unreasonable long waiting list for patients. Thus, stem cell-based therapeutic strategies may eliminate or lower the severity of immunosuppression through autologous or stem cell microencapsulation, both promising approaches for T1D cell therapy. In the cell microencapsulation strategy, insulin-producing cells are loaded into a semipermeable capsule made from biodegradable materials that can allow for the secretion of insulin while providing the cells with nutrients and protection from the host immune system.[20,21]

A relevant NHP model to address these clinical outcome concerns is crucial. A NHP model of T1D, the streptozotocin (STZ) NHP model of T1D, has been developed.[22] In this animal model, arterial and venous tether catheters enable continuous measurement of glucose, C-peptide, and other various biochemical measurements. STZ is administered intravenously to ablate the pancreatic β-cells and to induce diabetes. In this model, a comprehensive therapeutic strategy was implemented including a tether system, permanent indwelling catheter implants, an optimal hydration protocol, and pain and anxiety management. Glucose levels post-STZ administration were monitored moment by moment with continuous intravenous insulin therapy. Among the advantages of the STZ NHP model of T1D is that clinically relevant doses of stem cells and routes of delivery may be tested.

A recent study in this STZ-baboon model used an ultrasound-targeted microbubble destruction approach, in which nonviral gene therapy was targeted to pancreatic islets. STZ hyperglycemic-induced conscious tethered baboons received a gene cocktail comprising *cyclin D2*, *CDK*, and *GLP1*, which normalized intravenous glucose tolerance test curves. Immunohistochemistry demonstrated evidence of islet regeneration and restoration of β-cell mass.[22] STZ targets GLUT2 glucose transporter on β-cells and ablates them through the release of reactive oxygen species and alkylation of DNA.[23] Despite the ability to destroy β-cells, STZ is not necessarily representative of the autoimmune condition seen in T1D.[24] In this regard, transgenesis in NHPs[25,26] may play an important role in developing relevant models that closely mimic the autoimmune component of T1D.

Human pluripotent stem cells have indisputable cardiomyocyte (CM)-generating abilities and have been extensively investigated for repair of the injured heart. Studies of human embryonic stem cell-derived CMs (hESC-CMs) in small animal models have shown favorable effects of stem cell therapy. The human heart has a limited capacity for regeneration after injury. NHP models of myocardial infarction are well established and essential for addressing critical preclinical questions before the initiation of clinical trials. Myocardial infarction is induced in NHP using a coronary catheter that engages the main coronary artery, a guide wire, and an angioplasty balloon. The angioplasty balloon is inserted into the anterior descending artery and inflated to block circulation for a predetermined period, usually 90 min followed by reperfusion. This animal model has been used to evaluate hESC-CMs and reprogramed somatic cells (iPSC) for their regenerative capacity of the heart.[27-29]

The characterization of an NHP model of focal ischemia reperfusion with a defined syndrome, impaired arm function, and finger dexterity in the context of the current NHP models has recently been reported.[30] In this review, we take the opportunity to address another relevant model, that is the transient global ischemia (TGI).[31,32] Noting its difference from the clinical condition of brain ischemia, the induction of the TGI model may be a more appropriate model for cardiac arrest. However, the cell death events that precede an initial blood flow disruption are indicative of stroke-induced cell loss in distinct brain regions, particularly the hippocampus. Furthermore, the behavioral deficits evaluated in TGI animal models, such as memory decline, mirror the cognitive impairment assessed in clinical stroke patients.[33] The NHP model offers numerous opportunities, particularly the applicability to cerebral ischemia pathology.

NHP models can also offer important information for stroke rehabilitation that complement findings from rodent models. In a recent set of guidelines, the Stroke Therapy Academic Industry Roundtable (STAIR) has emphasized the

necessity for NHP models of stroke for preclinical progress of neuroprotective therapies.[34] These guidelines were prompted by effective results in the laboratory that failed to produce beneficial results in the clinic. Certain features of stroke are consistent only with humans and NHPs, including the subcortical white matter injury and the internal capsule where the descending cortical pathways are found. Likewise, rats do not display the advanced development of the direct cortico-motorneuronal pathway, which dictates the precise motor skills impacted by stroke. Recent studies have described NHP models of stroke with excellent imaging, behavioral, and pathophysiological characterization[35-41] and have demonstrated the value of these models in preclinical advancement of therapeutic mediation.[42-44] The goal of NHP models is to accurately predict results, identify challenges, and increase the probability of successful therapy in human clinical trials. Various NHP species have demonstrated and validated the presence of ischemic neuronal loss in discrete regions of the brain after focal or global ischemia, and thus they would be crucial in understanding the pathophysiology of stroke and in stem cell translational research.

Guidelines Proposed to Enforce Uniformity and Advance Further Research

Stem cell Therapeutics as an Emerging Paradigm for Stroke (STEPS)[45-48] has established guidelines that will provide the foundation for investigating the translation of cell-based therapeutics from the laboratory to the clinic by using animal stroke models. As emphasized in the STEPS, testing the potential of anti-stroke therapeutics is critically dependent on the appropriate use of species and applicable stroke animal models, such as NHPs. While larger animals are favorable, the lack of a reputable NHP stroke model leads to an emphasis on safety rather than efficacy as an appropriate outcome measure. Although the STEPS welcomes different, available types of surgical methods, it highlights the importance of the "end point" rather than the "technique employed" to produce the stroke. The conditions of the stroke model should mirror the human disease state very closely to improve the success of translating therapeutics from the laboratory to the clinic.[46,47]

A standard-of-care therapy (i.e., tissue plasminogen activator (tPA), rehabilitation therapy) must be established as a control group for translational studies. Currently, small animals such as rodents and rabbits receive such "best in class" control, yet accommodating this treatment to NHPs remains a challenge. The major obstacle in extending such control group to NHPs is the difficulty of standardizing the tPA treatment and rehabilitation therapy in these larger animal models. A solution to this would require NHP research personnel with expertise in this specific treatment and therapy.

Consideration must be given to the experimental therapeutic mechanism of action (MOA) in choosing the potential therapeutic windows for the various species utilized for stroke modeling. Subsequently, the stroke model and the species will be chosen that are deemed suitable to examine the MOA. For example, if the neuroprotective stage is targeted, the therapeutic window is expected to be during the acute stage of stroke. On the other hand, if the therapeutic MOA targets the neurorestorative stage instead, the window will most likely be within the subacute and chronic stages of the stroke. Small species have generally been utilized for examining therapeutic windows ranging from acute, subacute, and chronic stages of stroke. The use of smaller species can be attributed to the much less laborious postoperative care. Similarly, to humans and depending on the extent of the lesion, larger animal models of stroke, including NHPs, may require extensive postoperative monitoring and care. In addition, further studies are required to validate the standardization of NHP stroke models, particularly the chronic stage. This may restrict their use in examining therapeutic windows during the acute and subacute stages of stroke.

The NIH's National Institute of Neurological Disorders and Stroke has advanced the integration of RIGOR guidelines for translational research, along with recommendations by STAIR and STEPS, to standardize stroke research procedures worldwide. The STAIR, STEPS, and RIGOR guidelines are similar in highlighting the importance of good laboratory practices (GLPs), such as the requirement for all animal modeling studies to uphold treatment conditions such as blinding, randomization, consideration of sex and age variables, and complete power and statistical analysis. In addition, when proposing translational applications and manuscripts, one must clearly present these GLP practices.[48] Translational Stroke Research and the Journal of Neurology and Neurophysiology have recognized this policy in their submissions. Although many animal models exist, finding optimal animal models is key to enhance the successful translation of novel therapeutics from the laboratory to the clinic. Equally significant to this is asserting a transparency of any person's conflict of interest that may possibly suggest bias in the study.[49]

Future Direction for Nonhuman Primate Models in Translational Medicine

Given their close similarities to humans, the NHP model offers unique opportunities to understand biological mechanisms and translational applications with direct relevance to human conditions. Still, the value of using these primates must be carefully assessed, taking into account the expense of specialized equipment and requirement of highly specialized personnel. As such, carrying out initial fundamental studies in small animal models such as rodents may prove to be more efficient before translating such research to NHP models and eventually to first-in-human trials.

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Conflicts of interest
There are no conflicts of interest.

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