OPEN ACCESS
J. Adv. Vet. Anim. Res., 1(3): 75-85.
Available at- *http://bdvets.org/JAVAR*
JAVAR Journal of Advanced Veterinary and Animal Research
REVIEW ARTICLE
Volume 1 Issue 3 (September 2014)
DOI: 10.5455/javar.2014.a17

Stem cell therapy in animal models of central nervous system (CNS) diseases: therapeutic role, challenges and perspectives

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ABSTRACT

Many human diseases relating to central nervous system (CNS) are mimicked in animal models to evaluate the efficacy of stem cell therapy. The therapeutic role of stem cells in animal models of CNS diseases include replacement of diseased or degenerated neuron, oligodendrocytes or astrocytes with healthy ones, secretion of neurotrophic factors and delivery of therapeutics/genes. Scaffolds can be utilized for delivering stem cells in brain. Sustained delivery of stem cells, lineage specific differentiation, and enhanced neuronal network integration are the hallmarks of scaffold mediated stem cell delivery in CNS diseases. This review discusses the therapeutic role, challenges and future perspectives of stem cell therapy in animal models of CNS diseases.

Keywords

Animal models, Cell replacement, Challenges, CNS diseases, Scaffold, Stem cell therapy

Received 02 May 2014, **Revised**: 20 May 2014,
Accepted : 21 May 2014, **Published online**: 03 June 2014.

INTRODUCTION

Treatment of central nervous system (CNS) injuries had been a challenge for medical and veterinary clinicians. It was in the naïve stage until early 20th century (Schmidt and Leach, 2003) as the recovery in CNS is limited by the insufficient self–repair and regeneration abilities of the brain tissues (Bjorklund and Lindvall, 2000). Main challenges in the treatment of brain diseases include blood brain barrier (bbb) with tight intercellular junctions, and absence of fenestrations (Brightman and Reese, 1969). These prevent the uptake of majority of therapeutics (Pardridge, 2003), active drug efflux pumps in the bbb (Golden and Pollack, 2003), which pumps out the drugs from the brain, high intercellular fluid pressure due to space occupying lesions that limits diffusion (Ali et al., 2006; Navalitloha et al., 2006), and the sensitivity of brain tissue that emphasizes the need for appropriate and precise dosing of chemotherapeutic agents (Roger et al., 2011).

Unrelenting reports on therapeutic uses of stem cells for CNS diseases have led to their wider acceptance and importance in the present scenario. According to the early school of thoughts, neurons of adult CNS of mammals have limited regeneration capacity, but later studies have confirmed that subgranular zone and dentate gyrus of hippocampus and lateral ventricles of forebrain are regions of potential neurogenesis in adult mammalian brain (Kempermann and Gage, 1999; Gross, 2000; Lie et al., 2004). This endogenous regeneration potential of CNS could be stimulated to aid the repair of damaged brain tissue (Nakatomi et al., 2002). Even from the areas of adult CNS where neurogenesis is not apparent, stem cells and their

progenitors can be extracted, expanded and differentiated into neurons and glia *in vitro* (Aboody et al., 2011), which could be later implanted *in vivo*. Innumerable CNS diseases including stroke, brain tumors, epilepsy, Parkinson's Disease (PD), Huntington's disease (HD), Alzheimer's disease (AD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and spinal cord injuries could benefit from stem cell therapy (Chu et al., 2004; Carri et al., 2006; Ziv et al., 2006; James and Cavenne, 2009; Jiang et al., 2011; Cohen, 2013; Kim et al., 2013; Roper and Steindler, 2013; van Gorp et al., 2013; Mochizuki et al., 2014).

The term stem cell is a broader concept. The stem cell types which are used for treating CNS diseases include adult neural stem/neural progenitor cells, bone marrow derived mesenchymal stem cells, adipose derived mesenchymal stem cells, umbilical cord blood mesenchymal stem cells, embryonic/fetal mesenchymal stem cells, and induced pleuripotent stem cells (Chu et al., 2004; Ziv et al., 2006; Chen et al., 2010; Jiang et al., 2011; Gu, 2013; Razavi et al., 2013; Yang et al., 2013). Among the various types of stem cells, induced pleuripotent stem lines are derived from reprogramming the adult somatic cells to an embryonic stem cell state. They have proved themselves to be a potential autologous source of stem cells (Hu et al., 2010), and could be differentiated even to neurons (Kuo and Lin, 2013). The therapeutic role of stem cells in animal models of CNS diseases include cell replacement, as vehicle for delivery of genetically engineered genes and drugs (Roger et al., 2011), release of neurotrophic factors and vasoactive factors like anti–inflammatory cytokines which provide neuroprotection (Martino and Pluchino, 2006). This is only a bird's eye view of the topic. Here, the role of animal models seems to be noteworthy in that the therapeutic efficiency of stem cells for human CNS diseases is primarily evaluated by conducting laboratory animal trials.

STEM CELLS FOR CELL REPLACEMENT

Neural stem cells (NSCs) are the most logical stem cell type to be scrutinized in neural tissue engineering as they have the ability of self-renewal, and can be differentiated into neurons, astrocytes, and oligodendrocytes (Zhao et al., 2013). A schematic representation of their differentiation potential is given in Figure 1. NSCs are mostly harvested from subventri-cular zone (SVZ) region of brain and if meant for generation of dopaminergic neurons, from the ventral midbrain (Gu, 2013). ALS is a neurode-generative disease of the CNS causing abnormal function and degeneration of motor neurons in human spinal cord, cerebral cortex and brainstem resulting in rapidly progressing muscle weakness and death due to respiratory failure in a few years (Lunn et al., 2011; Vishwakarma et al., 2013). ALS may benefit from stem cell therapy especially in the earlier stages of disease by providing support and enrichment to existing motor neurons (Lunn et al., 2011) along with the concurrent replacement of lost motor neurons (Thonhoff et al., 2009).

Multiple sclerosis (MS) is a chronic inflammatory disease of CNS resulting in symptoms like musculoskeletal weakness, cognitive impairment which are sequale to axonal demyelination. Oligodendrocytes are the glial cells in CNS concerned with myelination of axons and hence stem cell derived oligodendrocytes could be a promising therapeutic option for MS. Research has shown that NSCs upon differentiation into oligodendrocytes can remyelinate axons in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis (Pluchino et al., 2003). NSCs could differentiate into cholinergic neurons, astrocytes, and oligodendrocytes and helped in amelioration of the learning/memory deficits in animal models of AD (Abdel–Salam, 2011). PD is a progressive, idiopathic neurodegenerative disorder of the CNS where there is dysfunction and loss of dopamine secreting neurons in the substantia nigra, leading to the characteristic symptom of debilitating motor impairments. Stem cell therapy could aid in its cure by serving as a source of dopaminergic neurons (Emerich et al., 2013). When dopaminergic neurons generated from stem cells were transplanted into primate models of PD, it diminished symptoms observed in this neurodegenerative disorder (Manganas and Maletic– Savatic, 2005).

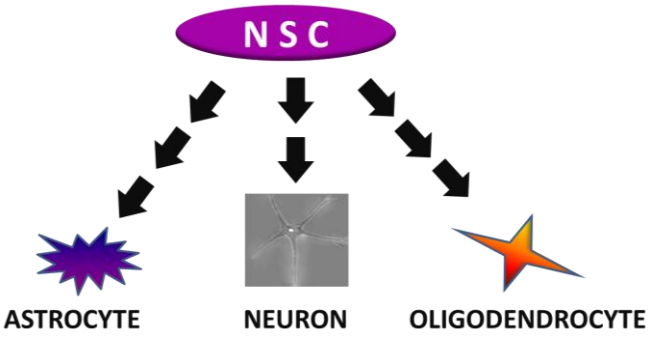

Figure 1: Differentiation potential of NSC.

Stem cells have found a role in successful treatment of stroke as there are reports on migration of neural progenitor cells (NPC) towards the lesion with formation of new neurons (Kelly et al., 2004), and reestablishment of neural connections with functional recovery (Hayashi et al., 2006). When human embryonic stem cell derived oligodendrocyte progenytors and motor neuron progenitors were transplanted into the transected spinal cord of adult rats immediately after the injury, they could differentiate into oligodendrocytes, astrocytes and neurons. In addition to this, there was improvement in locomotor functions without teratoma formation (Erceg et al., 2010). This in turn corroborates cell replacement role of stem cells in spinal cord injury. Though the bone marrow stem cells could give rise to only a lesser proportion of neuron like cells in comparison to brain derived neural stem cells, they could be an assuring therapy for CNS injury and neurodegenerative diseases (Song et al., 2007).

STEM CELL MEDIATED GENE THERAPY

Gene therapy is the concept and procedure for transfer of therapeutic genetic material into the cells to cure diseases (Moirano and Emburg, 2006). Using stem cells, an ex vivo gene therapy is performed, which means that the genetic material is transferred into the cultured cells prior to transplantation (Loscher et al., 2008). In ex vivo gene therapy, mostly embryonic stem cells or neural stem cells are used owing to their expanding capabilities and differentiation potential to various types of neural cells. However, the chances of genetic incorporation into the brain to generate the desired neural phenotype is limited (Van Dycke et al., 2011). Genetically engineered stem cells have proven as useful in animal models of Parkinsonism (Anton et al., 1994); brain ischemia, spinal cord injury (Park et al., 2006; Kusano et al., 2010); gliomas (Aboody et al., 2000), ALS (Suzuki et al., 2007), and HD (Olson et al., 2012).

Genetic modification of NSCs with neutrotrophin-3 (NT–3) has been reported to promote myelination and to reduce astroglial scarring after transplantation in rodents with either injury of spinal cord or ischemic brain injury (Park et al., 2006; Kusano et al., 2010). Transplantation of genetically modified embryonic stem cell derived cells overexpressing neuroprotective factors results in functional recovery in animal models of ischemia (Shinozuka et al., 2013). Genetically engineered stem cells expressing cytokines have reported promising results in glioma models following intracranial administration (Ehtesham et al., 2002; Yang et al., 2004; Yuan et al., 2006). GDNF (glial cell derived neurotrophic factor) – over expressing neural stem/precursor cells delayed the degeneration of motor neurons in the spinal cord of rat model of ALS (Suzuki et al., 2007); whereas, they increased the survival of neuronal cells for up to 3 months post–transplantation in the striatum of presymptomatic transgenic mouse model of Huntinton's disease (Ebert et al., 2010).

Huntington's disease is caused by mutation of gene coding for protein mHTT (mutant huntingtin protein) resulting in cellular toxicity. Research in several HD animal models had shown that neuronal survival could be prolonged by enhancing the degradation/clearance of this protein from affected neurons. Patient derived induced pleuripotent stem cells (iPSC) were used for studying gene manipulation strategies for achieving this. Genome editing approaches directly targeting DNA for reducing mHTT protein has shown success in patient iPSC– derived neuronal models. But this has to be validated further in *in vivo* models (Yu et al., 2014). Small interfering RNAs can reduce mHTT and studies regarding safety and efficacy of siRNA delivery system using human MSCs are underway (Olson et al., 2012).

RELEASE OF TROPHIC FACTORS AND OTHER PARACRINE EFFECTS OF STEM CELLS

MSC and NPC secrete immune modulatory or neurotrophic paracrine factors which may have therapeutic benefits in treating experimentally induced CNS diseases in animal models (Drago et al., 2013; Lavoie and Rosu–Myles, 2013). In experimental studies of PD, NPC transplants secreting GDNF, and vascular–endothelial growth factor (VEGF) have shown positive outcome and are being assessed in pre–clinical trials for the treatment of the disease (Akerud et al., 2001). Upregulation of stromal cell-derived factor-1 (SDF–1), VEGF, and transforming growth factor beta (TGF β) were noticed in MSC transplanted spinal cord injury models of beagle dogs (Jung et al., 2009). Many recent studies focus on utilizing paracrine effects of stem cells in the therapy of CNS disease. Here, instead of going for implanting stem cells, the biologics secreted by stem cells termed as 'secretome' are used for repairing injured brain (Drago et al., 2013).

Human umbilical cord blood–derived mesenchymal stem cells delivered intracranially, in a mouse model of AD, improved spatial learning and memory decline possibly by neuroprotective effect (Lee et al., 2012). Yang et al. (2013) reported that single intracerebral injection of neuron–like cells, differentiated from human umbilical cord derived mesenchymal stem cells

(hUMSC–NC) could ameliorate memory deficits in mouse model of AD by alternative activation of microglia cells. These "alternatively activated" microglia (M2–like microglia) played protective roles in AD by phagocytizing Aβ (amyloid β–peptide – the key pathogenic factor of AD and reducing neuroinflammation. Yang et al. (2013) here emphasizes the paracrine effects of transplantation of hUMSC–NC. The paracrine effect has resulted from increased expression of an antiinflammatory cytokine namely, IL–4, which in turn led to M2–like microglial activation. As already mentioned, elevated amyloid β–peptide deposition is the key pathogenic factor for neuronal loss in AD. In another study utilizing human umbilical cord derived mesenchymal stem cells (hUMSC) in beta–amyloidosis mouse model of AD, amyloid plaques were reduced by secretion of a soluble intercellular adhesion molecule–1 (sICAM–1). This molecule exerted its effect by inducing Aβ degrading enzyme. This again outlines the paracrine mode of action of hUMSC (Kim et al., 2012).

STEM CELLS AS VECTOR FOR DRUG DELIVERY

Stem cell therapy combined with nanotechnology could be a promising tool to efficiently deliver drugs to brain tumors (Roger et al., 2011). Glioblastoma, is a lethal malignant tumor where even the standard protocols like surgical resection followed by concomitant chemotherapy and fractionated radiotherapy (Stupp et al., 2005; Stupp et al., 2009) could only prolong the life span by near about one year. Advances in the field of nanotechnology have led to the development of nanoparticles loaded with chemotherapeutics. The therapeutic agent is entrapped in, adsorbed or chemically coupled onto the nanoparticle surface. By this technique the therapeutic agents are protected from enzymatic and chemical degradation, thereby ensuring it's sustained and controlled release (Roger et al., 2011).

Stem cells can be used to carry these drug bound nanoparticles (Figure 2) to the lesion site. Neural stem cells, owing to their tropism towards glioma cells and ability to cross bbb, are excellent carriers for cytokines, viral particles and prodrugs (Aboody et al., 2000). Mesenchymal stem cells also have homing properties around glioma which could be utilized for glioma therapy. This homing is due to mechanisms mediated by several factors like epidermal growth factor (Sato et al., 2005), SDF–1 (Wynn et al., 2004), platelet–derived growth factor (Fiedler et al., 2002), matrix metalloproteinase–1 (Ho et al., 2009), and macrophage chemoattractant protein–1 (Xu et al., 2010). First step in stem cell mediated delivery of drug loaded nanoparticles is the incorporation of chemotherapeutic loaded nanoparticles into the stem cells in–vitro either spontaneously or via passive/active endocytosis. Secondly, these stem cells are injected intracranially into the tumor mass. The nanoparticle loaded stem cells will localize in the border between tumor cells and normal brain parenchyma and slowly release the chemotherapy drugs as depicted in Figure 2. This concept is already demonstrated by Roger et al. (2011), using MIAMI (Marrow–Isolated Adult Multilineage Inducible) cells, a subpopulation of human MSCs. NSC mediated delivery of secreted soluble variant of TRAIL, [Tumor Necrosis Factor –related apoptosis–inducing ligand (TRAIL) can selectively induce apoptosis in glioma cells] in combination with therapeutics like proteasome inhibitor, bortezomib (Balyasnikova et al., 2011) and kinase inhibitor, PI–103, in mice models of glioma (Bagci–Onder et al., 2011) increased survivability of mouse by inhibition of tumor growth and proliferation. This is yet another example of the role of stem cell as vector.

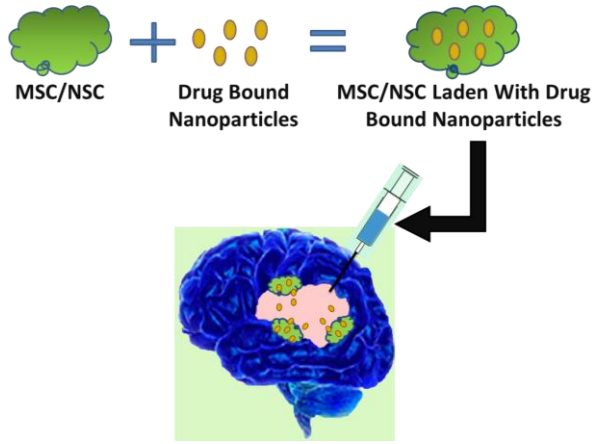

Figure 2: Stem cells acting as vector to release drug bound nanoparticles to the site of brain lesion.

Stem cells also have a role in treatment of epilepsy by delivery of adenosine (Van Dycke et al., 2010), which is a purine ribonucleoside with neuromodulator and neurotransmitter functions (Sachdeva and Gupta, 2013). Antiseizure and neuroprotective potentials of adenosine are known for long (Lee et al., 1984; Fredholm, 1997). The role of adenosine in seizure (Van Dycke et al., 2011) results from its binding to the presynaptic A_1 receptors which inhibits the release of excitatory neurotransmitters like glutamate. Systemic use of adenosine has severe side effects like decreased blood pressure and heart rate which emphasizes the

need for its local delivery into the brain via stem cells (Boison, 2005). According to Van Dycke et al. (2010), astrocytes derived from NPC and undifferentiated NPC of adenosine kinase deficient mice released therapeutically relevant amounts of adenosine under in vitro conditions. In case of brain tumors therapeutic delivery may be needed for short time duration only whereas epilepsy, which is a chronic disorder, needs lifelong local delivery of therapeutics. In the latter context, cell or gene therapy sounds theoretically a successful strategy, as long term release can be ensured without replacement or refilling (Van Dycke et al., 2011).

SCAFFOLDS FOR STEM CELL DELIVERY IN CNS DISEASES

Scaffolds aid cell proliferation and differentiation, by allowing diffusion of nutrients and exerting mechanical and biological influences on the cell. Delivery and duration of action of stem cells could also be prolonged by use of suitable scaffolds. In addition to these, they can be used for sustained release of lineage–specific inductive factors or small interfering ribonucleic acids (siRNAs) which can act as molecular mediators of neuronal differentiation. The efficacy of transplantation of NPC can be improved in CNS injury by the co–administration of biomaterial scaffolds (Potter et al., 2008). Biomaterials made of nanofibers, nanotubes and nanoparticles have been widely used in manipulating the fate of stem cells (Zhao et al., 2013). Carbon nanotubes can provide support, direct the differentiation of stem cells to neural lineages and promote signal transmission among neurons (Pastorin, 2011).

A nano–biohybrid system created by NSC progeny and graphene showed that graphene films can not only support neural network without affecting its structure and function but also could amplify the network activity and efficacy of neural signals (Tang et al., 2013). In ICC construct, i.e., inverted colloidal crystal scaffold comprising chitin, chitosan and gelatin, mouse iPSC can remain viable. Also it can accelerate differentiation of iPSC to neurons (Kuo and Lin, 2013). The knowledge of down regulation of RE 1 silencing transcription (REST) factor upon differentiation to neurons in NPC can be utilized to enhance in vitro neuronal differentiation of stem cells. Low et al. (2013), investigated the possibility of scaffold mediated gene silencing by delivering small interfering RNA/ transfection agent complexes via mussel– inspired polydopamine modified electrospun polycaprolactone nanofibre scaffolds and concluded that it could be a future promise for therapy as enhanced neuronal commitment of primary mouse neural progenitor cells and decreased glial cell differentiation was seen.

CHALLENGES AND PERSPECTIVES

The main limitations of stem cell therapy are with regard to the higher cost of commercialization, and the difficulties in approval of clinical trials. Another major problem related to NSC mediated regeneration in CNS trauma is due to the upregulation of inhibitory immune factors around the site of lesion resulting from the inflammatory process that ensues (Dooley et al., 2014). A recent study by Kyritsis et al. (2014) suggests that in mammals, acute inflammation is followed by chronic inflammation, which prevents functional recovery of brain tissue. Survival of the implanted differentiated neurons is to be ensured after transplantation for successful outcome. In ALS, though it had been shown that motor neurons derived from stem cells can be grafted safely without any rejection, the microenvironment remains hostile for their survival because of neuroinflammation, oxidative stress and glutamate excitotoxicity (Thonhoff et al., 2009).

As dysfunctional astrocytes also have a role in the survival of dying motor neurons in ALS, new studies are aimed at transplanting stem cell derived astrocytes for protecting the diseased motor neurons (Lindvall et al., 2012). The beneficial effects of NSC transplantation are limited by the unfriendly microenvironment at the site of CNS injury/degeneration (Lu et al., 2011). Hence stem cell transplantation together with enrichment of microenvironment with trophic factors is under investigation. In vivo tumorigenic potential of mesenchymal stem cell exosome (Zhu et al., 2012), NSC (Shinozuka et al., 2013), embryonic stem cells (Thonhoff et al., 2009; Mothe and Tator, 2012) and induced pluripotent stem cells is another impeding factor in progress. However, many studies with the above stem cell sources have shown positive therapeutic outcomes without tumor formation. Further research on tumorigenic potential of stem cells revealed that the presence of undifferentiated pluripotent cells as contaminants in neural committed transplants is the cause for teratomas. This tumor transformation can be halted by incorporating suicidal gene into these stem cells.

It is important to rule out any existing tumors by functional imaging modalities before stem cell infusion into brain or spinal cord (Olson et al., 2012). The homing tendency of MSC to hypoxic regions around tumor margins and its potent revascularization potency

has the flaw of supporting the survival of existing tumors, if given alone. A dark side to the use of human embryonic stem cells is the activation of immune responses. A way out has evolved by short term immune dampening treatment of these cells which could prevent post–transplantation rejection (Mochizuki et al., 2014). Undifferentiated iPSC are also immunogenic but are seldom used in transplantation studies without differentiating them into desired cell lines (Yamanaka, 2012).

Presently preclinical cell therapy trials are being carried out in animal models of CNS diseases. Though the predictive clinical outcome of such therapies seems to be encouraging, controlled clinical trials in large animal models are to be undertaken before evaluating the feasibility of stem cell therapy in human beings. Even in those CNS diseases where the clinical phase trials have started or culminated the diagnostic techniques for accurately predicting the cell integration and survival in live patients is underway. A solution to this has evolved from the finding that NSCs may be labelled with superparamagnetic iron oxide before administration and their distribution in the body can be noninvasively monitored by MRI. This is awaiting FDA approval for implementation in clinical trials (Aboody et al., 2011).

Certain other issues need to be addressed in the future and one of them is how the survivability of transplanted stem cells can be augmented. Studies have shown that the survival, growth and function of the neurons can be enhanced by trophic protein factors and hence combining differentiated neurons with these factors could be a concept for future research (Emerich et al., 2013). The optimal dose of stem cells to be used, route of administration and sex of the donor/recipient vary with the stem cell type being used (Shinozuka et al., 2013) and future trials should be aimed at standardizing these factors with regard to different stem cell types for various CNS diseases. When to give stem cell therapy after disease onset is yet another issue. Most of the CNS diseases that gained benefit from stem cell therapy had shown that an early intervention is necessary for successful outcome. Though there are reports of improvement of locomotor function following immediate stem cell therapy, optimal time period for stem cell transplantation following spinal cord injury is 1–2 weeks after injury. The immediate post–traumatic microenviroment does not support the survival and differentiation of neural stem cell/progenitor cells. In chronic stage, there is glial scar formation at the site of injury which inhibits axonal regeneration (Nakamura and Okano, 2013).

A recent progress in the field of cell therapy is the invention of technology for cell reprogramming and development of iPSC. Induced pluripotent stem cell lines derived from patients suffering from PD, AD, Autism, Rett syndrome (a paediatric neural development disease) and Schizophrenia are now used as cell models for studying pathogenesis, and to develop assays for drug discovery (Yuan and Shaner, 2013). They can serve as autologous stem cell sources with more controlled methods of reprogramming. This new technology provides scope for a diversion from the use of animal models as a primary step for lab evaluation of new treatment strategies.

CONCLUSIONS

The therapeutic effects of stem cells in animal models of CNS diseases are discussed in this review. Majority of the research outputs from different laboratories are encouraging and these results are extrapolated to predict the possible outcome in human beings. In this context, one main lacuna is that there is absence of complete recapitulation of the human CNS disease in these lab animal models. Here, we conclude this review by stating that research in animal models of CNS disease is currently in progress for developing new treatment options of which stem cell seems to be promising. The broad arena of stem cell biology in neuroscience provides an ample scope for future research in pet animal diseases like CNS related paralysis/seizures.

ACKNOWLEDGEMENT

The authors are thankful to the Director, Indian Veterinary Research Institute for providing necessary facilities for compiling this review article.

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