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Applications of Stem Cells in Veterinary Medicine

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ABSTRACT

The stem cell field in veterinary medicine continues to evolve rapidly both experimentally and clinically. Stem cells are most commonly used in clinical veterinary medicine in therapeutic applications for the treatment of musculoskeletal injuries. New technologies of assisted reproduction are being developed to apply the properties of spermatogonial stem cells to preserve endangered animal species which can also be used to generate transgenic animals for production of pharmaceuticals or for use as biomedical models. Small and large animals serve as valuable models for preclinical evaluation of stem cell applications in human beings and in veterinary, in areas such as spinal cord injury and myocardial infarction. Reviews on the use of animal models for stem cell research have been published recently. Therefore, in this overview, animal model research will be reviewed only in the context of supporting the current clinical application of stem cells especially in veterinary medicine.

1. Introduction

Stem cells are defined as unspecialized cells having the capacity of self-renewal by cell division sometimes even after long periods of inactivity, to proliferate extensively into one or more cell/tissue types (Gade *et al.,* 2012 a, b). The rigorous definition of a stem cell requires that it possesses two properties: self renewal and unlimited potency. Self renewal means the ability to go through numerous cycles of cell division while maintaining the undifferentiated state. This unique potential of these cells makes their application indispensable in the area of regenerative therapeutics. Stem cells are undoubtedly, most promising for the cell-based therapies that are currently tested in pre-clinical trials for a wide range of ailments (Gade *et al.,* 2012 a,b) like leukemias, lymphomas, solid tumors and nonmalignant disorders (Gratwohl *et al.,* 2008; Koch *et al.,* 2009). Multipotent mesenchymal stromal cells or mesenchymal stem cells (MSCs) have not yet reached the mainstream clinical practice, but however it has shown promising results with huge future scope in veterinary medicine.

Types of stem cells

Stem cells can be categorized broadly as embryonic stem cells (ESC), adult or tissue-specific stem cells (ADC) or induced pluripotent stem cells (iPSCs). These can also be classified according to their potency as unipotent, multipotent, pluripotent and totipotent stem cells. Adult stem cells are derived from adult body organs whereas embryonic stem cells are drawn from embryos. Embryonic stem cells obtained from an early stage embryo are totipotent, however, stem cells isolated from a hatched blastocyst are pluripotent. Pluripotent stem cells have inherent capacity of differentiation to cells of the three somatic germ layers (ectoderm, endoderm and mesoderm). ESCs may be derived from morulae, intact blastocysts, inner cell mass, single blastomeres or even from parthenogenetic embryos (Sritanaudomchai *et al.,* 2007). Adult stem cells are found in various tissues of adults including bone marrow and contribute to tissue regeneration during adult life (Amarpal 2008) and include hematopoeitic stem cells, mesenchymal stem cells, neural stem cells, skin stem cells, retinal stem cells *etc.*

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MSCs have been most widely used for research and therapy in experimental, preclinical and clinical trials (Lee 2008) and are most abundantly isolated from bone marrow, fat, umbilical cord blood, amniotic fluid, dental pulp, tendons, synovial membrane, skeletal muscle and Warton's jelly (Xu *et al.,*2005; Pratheesh *et al.,* 2013). MSCs can differentiate into cells of mesodermal origin *viz.* adipocytes; osteoblasts and chondrocytes; tenocytes; skeletal myocytes and visceral stromal cells.

Stem cell products in clinical use

In veterinary medicine, three MSC-based approaches are currently used for the treatment of tendon, ligament or cartilage/joint injuries in horses or dogs. The first MSC-based method relies on a culture-expanded cell population derived from bone marrow aspirate, the second is another bone marrow aspirate-based approach using a concentrated mixed cell population derived from bone marrow aspiration and the third method employs a mixed nucleated cell population derived from adipose tissue (Fortier and Travis 2011).

Culture-expanded bone marrow-derived mesenchymalstem cells (BM-MSCs)

Bone marrow-derived mesenchymal stem cells (BM-MSCs) have the advantages of being easily and relatively non-invasively obtained and have a greater capacity of differentiation in comparison with other MSCs (Vidal *et al.,* 2008). Furthermore, BM-MSCs have received the most scientific attention hence are the best characterized. One disadvantage of culture-expanded BM-MSCs is the time lag of 3 to 6 weeks from bone marrow aspirate until treatment. This time lag is necessitated by the time required to grow the MSCs. Bone marrow is typically aspirated from the sternum proximal humerus, proximal femur, or tuber coxae.

Bone marrow concentrates (BMC)

Concentrated bone marrow aspirate was designed to increase the concentration of stem cells, the concentrations of platelets and anabolic growth factors (Fortier *et al.,* 2010).When combine with thrombin, the fibrinogen present in BMC is converted to fibrin and a solid scaffold forms to retain the cells and growth factors in a given location.

Adipose-derived stem cells (ADSCs)

The currently available technique uses a mixture of cells derived from adipose tissue surgically excised from horses or dogs.

The ADSCs are simply isolated and injected without a cell culture step. Compared with cultured BM-MSCs, this technique has the advantage of supplying cells in a short time period (48 hours) and it should be remembered that although there are a large number of nucleated cells retrieved from the adipose digest, only a small percentage of nucleated cells are stem cells. In humans, 0.7% to 5% of nucleated cells in the stromal vascular fraction are stem cells (Jurgens *et al.,* 2008).

Applications

Wound healing

Several studies indicated that MSCs derived from the bone marrow could significantly impact wound healing in animals, through cell differentiation and release of paracrine factors. BM-MSCs have been shown to promote the healing of diabetic wounds (Gade *et al.,*2012 a,b). More recently, allogeneic BM-MSCs exhibited similar survival, engraftment and effect as syngeneic BM-MSCs in promoting wound healing. BM-MSC-treated wounds exhibited significantly faster wound closure, with increased re-epithelialization, cellularity, and angiogenesis. In addition to differentiating into keratinocytes and forming appendage-like structures, BM-MSCs in the wound enhance the proliferation of endogenous keratinocytes and increase the number of regenerating appendage-like structures (Wu *et al.,* 2010). Even xenogenic human MSCs were used for incisional wound healing and tissue regeneration in rabbit and sheep (Mackenzie and Flake 2001; Stoff *et al.,* 2009). In caprine, Wharton's jelly mesenchymal stem cells (WJMSCs) of umbilical cord were used to treat cutaneous wounds (Azari
*et al.,*2011).

Tendonitis

The use of culture-expanded BM-MSCs for the treatment of tendon injuries is supported by experimental investigations in horses and laboratory animals in which MSCs were implanted in surgically or collagenase-induced tendon lesions and shown favorable effects on tissue organization and composition (Schnabel *et al.,* 2009). More recently, a small case control study demonstrated that, as a result of BM-MSCs, 90% of treated horses successfully returned to pre-injury athletic function and race horses suffered no re-injury of the superficial digital flexor tendon after 2 years (Pacini *et al.,* 2007). When a natural mechanical stimulus is combined with both bone marrow and adipose derived stem cells, regeneration of tendon tissue is promoted along with restoration of natural movement. Bone marrow derived autologous MSCs along with collagen gel were used to repair the surgically induced patellar tendon defect in adult New

Zealand White rabbits, the treated group showed significant improvement in its biomechanical properties after 4 weeks post treament (Awad *et al.,*1999; Krampera *et al.,* 2006).

Cartilage injury/osteoarthritis

Culture-expanded BM-MSCs have been evaluated in an equine model of acute cartilage injury (Wilke *et al.,* 2007). A 30-day re-check arthroscopy scores and biopsy assessments for the BM-MSCs lesions were significantly better than fibrin-only control grafts. In an equine model of early osteoarthritis (OA), a direct comparison between BM-MSCs and adipose-derived stromal vascular fraction ADSCs was made (Frisbie *et al.,* 2009) and found that joints treated with BM-MSCs showed significantly less synovial effusion and significantly lower prostaglandina E2 (PGE2) concentrations in comparison with those treated with ADSCs indicating the potential use of stem cells for such type of illness (Najar *et al.,* 2010). In fact, ADSCs led to an increase in synovial fluid concentration of the pro-inflammatory cytokine tumor necrosis factor-alpha. In dogs, two reports of improved clinical signs of OA after treatment have been published. In a double-blinded study assessing the use of ADSCs in the hip joint of dogs, examining veterinarians reported signs of clinical improvement (Black *et al.,* 2007) In a second, uncontrolled study using ADSCs for elbow OA, reported improvements in clinical signs (Black *et al.,* 2008).

Bone repair

The MSCs can undergo osteogenic differentiation, and exploration to augment bone repair and regeneration (Amarpal 2008; Gade *et al.,* 2012 a, b; Udehiya *et al.,* 2013). MSCs stimulate new bone formation in areas of implant site indicating that either these cells were infiltrating the adjacent host bone or stimulating the host bone to regenerate new bone (Tolley *et al.,* 2004; Kraus and Kirker 2006). Among large-sized animals the use of sheep autologous BMSC in conjunction with hydroxyapatite ceramic (HAC)-based carriers results in faster bone repair (Kon *et al.,*2000). Goat bone marrow derived MSCs cultured with scaffolds could repair the segmental bone defect in the tibia by 8 weeks after surgery (Liu *et al.,* 2010).These reports demonstrate the feasibility and efficiency of using MSCs to augment the repair of bone defects in animals (Frisbie and Smith 2010). Autologous adipose derived stem cells (ADSCs) seeded on a composition scaffold made from hydroxyapatite (HA) and chitosan (CH) fibres has been successfully used for the treatment of non union of radius/ulna in a cross bred dog (Lee *et al.,* 2009).

Recent studies suggested that there are minimal chances of rejection or immunogenic reaction when allogenic mesenchymal stem cells are used and they are as effective as autogenic stem cells in the repair of experimental bone defects (Udehiya *et al.,* 2013).

Spinal cord injuries

Acute spinal injuries are common in canines and felines that lead to loss of tissue, including myelinated fibre tracts responsible for carrying nerve impulses. The nervous tissue has limited regeneration capacity and complete restoration of locomotor activity is a challenge to modern therapeutics. MSCs were found to have the ability to differentiate into oligodendrocytes and other cell types needed to restore neuronal function in injured spinal cord (Dobkin *et al.,* 2006; Harris 2008). Therefore transplantation of stem cells with the ability to differentiate into neurons and supporting cells may be practical method for recovery in such cases (Gade *et al.,* 2012 a,b). In addition to trans differentiation, they may secrete growth factors that could support neuro protection and/or axon regeneration. Bone marrow derived MSCs were first used in Rhesus monkeys for nervous tissue regeneration which appeared promising (Deng *et al.,* 2006). Xenogenic transplantation of human umbilical cord blood (UCB) stem cells in rats following spinal cord injury significantly enhanced locomotor function within 14 days after therapy (Dasari *et al.,* 2007). Intrathecal implantation of autologus bone marrow derived MSCs improved locomotor activity significantly in dogs within one week (Adel and Gabr 2007).

Ischemic brain injury

Neural stem cell therapy has raised the hopes in order to treat neurodegenerative diseases. In order to properly integrate in the brain cells that are injured, isolation as well as enrichment and propagation of neural stem cells are necessary. Damage caused by stroke injury to the central nervous system is a major cause of death and disability in humans. Transplantation of MSC directly into adult rodent brain was found safe and it reduced functional deficits associated with the stroke (Harris 2008) which supported that MSCs can adopt neural cell fates and are feasible candidates for the treatment of stroke injury (Burns *et al.,* 2009). Exogenous stem cells offer the complementary advantages of being available in unlimited numbers with additional control over fate, cell number, timing and site of delivery. The fact that substantial functional gains have been observed in animal models after delivery of cells of both neural and non-neural origin in preclinical models of ischemic brain injury is encouraging (Gage 2000). MSCs were also found useful for treating cerebral infarction and ischemia (Chen *et al.,* 2001).

Myocardial infarcts

In dogs, cardiac disease causes significant morbidity and mortality, contributing to over 50% of mortalities in some breeds such as the Cavalier King Charles spaniel. The stem cell therapy would minimize loss of cardiomyocytes by reducing cell death, promote the return of a stunned and hibernating myocardium to normal function, stimulate revascularization of the damaged region by enhancing angiogenesis and regenerate viable cardiomyocytes thereby preserving contractile function and reducing the opportunity for scarring (Caspi *et al.,* 2007). Adipose as well as bone marrow derived stem cells have successfully been used to treat myocardial infarction in dog (Gade *et al.,*2012 a, b). Before injecting into the heart, stem cells can be used for induction of a cardiac cell fate resulting in increased contractility of the heart along with reduction in the damaged area a few weeks after the application of stem cells (Black *et al.,* 2008). Use of autologous bone marrow stem cells for treatment of myocardial infarction is a novel application of stem cell therapy that is gaining popularity nowadays (Anderson 2008). Clinical experiences have shown that MSCs when therapeutically delivered improve function of heart after an acute myocardial infarction. This could be due to the fact that MSCs can generate various signalling molecules that are cardio-protective and can differentiate into a myocyte as well as into the lineage of the vascular system (Udehiya *et al.,* 2013).

Hepatic applications

The existence of liver stem cells within the adult bone marrow was first reported in 1999 and since then it had been confirmed in multiple further studies. MSC can be induced to a hepatic lineage by incubation with specific growth factors such as hepatocyte growth factor (HGF) and have been isolated from bone marrow, umbilical cord blood and adipose tissue. The effectiveness of systemically administered MSC in the repair and regeneration of liver tissue has been most extensively studied in the carbon tetrachloride (CCl4) model of progressive liver fibrosis in mice (Zhao *et al.,* 2005; Oyagi *et al.,* 2006).

Solid organ transplantation

The use of MSC for preventing acute rejection following solid organ transplantation may have significant advantages, as immune-suppression is coupled with the ability to repair ischemic damage and therefore MSCs transplantation has the potential to target both inflammatory and allo-immune pathways. MSCs exert an immuno-modulatory effect towards a large number of effector cells, including CD4+ and CD8+ T cells, NK cells, B cells, monocytes and dendritic cells (Peroni and Borjesson 2011). However, results on prolongation of graft survival have been conflicting. Advancement in the field of stem cell research has led clinical trials to treat kidney diseases in cats and liver diseases in dogs (Togel *et al.,* 2005; Quimby *et al.,* 2013). Placement of adult stem cell seeds inside a tissue bed soil allowing the stem cells to differentiate into the tissue bed cells is a possible method of tissue regeneration in adults. Investigation concerning soil tissue conducive to regeneration is however still in its infancy (Cyranoski 2009).

Leukemia

Allogeneic blood as well as marrow transplantation (allogeneic BMT) in order to treat malignant hematologic disorders and genetic diseases requires myeloblative conditioning using the radiation and immunosuppressive drugs. Allogeneic BMT for treating leukemia type of cancers induce immune-mediated graft-versus-leukemia (GVL) reaction, which is beneficial for eliminating the residual pathological host cells and curing the cancer. For this reason, relatively non-myeloablative conditioning before allogeneic BMT has been introduced in recent years for establishing host-versus-graft tolerance. It helps in engraftment of donor immuno-hematopoietic cells for introducing GVL effects. Thus, allogeneic non-myeloablative stem cell transplantation is potentially a new approach in human medicine to successfully eradicate malignant cancer cells as well as genetically abnormal host hematopoietic cells (Lin *et al.,* 1996; Slavin *et al.,* 1998).

Testis xenografting

The primary clinical application for testis xenografting would be as a means to preserve the breeding potential of a genetically valuable pre-pubertal male animal (Pukazhenthi *et al.,* 2006). If adult males die before contributing their genes to the population, mature sperm can be collected and cryopreserved for future use in artificial insemination or for of *in vitro* fertilization (IVF).By means of this approach, morphologically mature sperm have been produced in xenografts from a number of species, including rabbits (Shinohara *et al.,* 2002), pigs and goats (Honaramooz *et al.,* 2002), sheep (Zeng *et al.,* 2006), cats (Snedaker *et al.,* 2004) and dogs (Abrishami *et al.,* 2010). However, the efficiency of spermatogenesis in xenografts differs among species, with the bull (Rathi *et al.,* 2006), cats (Snedaker *et al.,* 2004) and dogs (Abrishami *et al.,* 2010) being less efficient. One common finding across species is that if the donor testis tissue has germ cells actively undergoing meiosis (as in puberty or adulthood), then the xenografts lose the ability to support spermatogenesis (Arregui *et al.,* 2008).

Because there is no epididymis in this system, the functionally immature sperm can help generate off spring only through intra cytoplasmic sperm injection (ICSI). Thus, although banking of material from genetically valuable individuals of multiple species might begin now, the ultimate production of off spring is restricted until ICSI is optimized for that species.

Spermatogonial stem cell transplantation

The primary clinical uses of SSCT would be to preserve or manipulate the male germline or both (Dobrinski *et al.,*2007). Briefly, the technique involves isolation of a mixed germ cell population from a donor testis and injected in a retrograde fashion into the testes of a recipient animal are often treated with focal testicular irradiation (Kim *et al.,* 2008) or systemic busulfan (Hill *et al.,* 2006) to reduce their endogenous SSC. Sometime is given for colonization, proliferation, and spermatogenesis, semen is collected and assessed for the relative percentage. This technique has multiple steps that are technically challenging, time consuming and labor-intensive. Therefore, it is likely to be used in the future primarily as a clinical tool to develop transgenic biomedical research models or for the production of transgenic farm animals that produce tissues/organs genetically engineered to be compatible across species or to produce pharmaceutical proteins (Houdebine *et al.,* 2009). Therefore, utilization for the conservation of threatened species would require not only the use of a suitable domestic animal recipient that would support spermatogenesis of the donor but also some method of sorting the sperm of donor origin from that of recipient origin.

Generation of transgenic animals

Isolation of totipotent stem cell from embryos and subsequent incorporation of the desired DNA into the embryo of the host results in generation of chimeric animals. A type of adult stem cells, spermatogonial stem cells can differentiate in the niche of a testis, which are used for the generation of transgenic animals by either transplantation or by directly injecting in the seminiferous tubules (Brinster 2002; Miao 2011). Blastocyst injection using transgenic pluripotent stem cells is also an alternative approach for the generation of transgenic animals.

Stem cell oriented products and application of gene therapy

There is every possible chance of curing a disease hypothetically if an individual is having a disease caused by single gene mutation using either their own stem cells following *in vitro* gene modification or allogeneic adult stem cells. Previously, somatic cell therapy and gene therapy were synonymous, but now they are well distinguished and the stem cell therapy without any genetic manipulation is more widely acceptable than the gene therapy approaches. Food and Drug Administration (FDA) gives approval to treatment of somatic cells manipulated *ex vivo* with a gene therapy vector and such a strategy find its relevance at present due to greater possibility of manipulating isolated population of stem cells in culture. The products that come under biological products are those containing genetically modified cells for transplantation as well as viral vectors (Maitra *et al.,* 2005). Gene therapy strategies have also aided to the treatment of intracranial tumors in dogs (Krishnamurthy *et al.,* 2009).

Future perspectives

In order to determine the safety and efficacy of stem cell based products, much has to be learned. The research and development should continue in this area to understand the several unknown molecular control and regulation in the life cycle of stem cells. The risk assessment of inappropriate cell functioning can be hastened provided the biology of self-renewal as well as differentiation is understood properly. Risk of carcinogenesis may be associated particularly with the induced pluripotent stem cells and embryonic stem cells. In order to ensure safety of stem cell therapy, non-invasive tracing of transplanted cells *in vivo* and development of techniques for identification of mixed population of cells in culture are critical factors. Ethical issues associated with harming of animal embryos and animal welfare also need to be given due consideration in the area of stem cell based disease modelling and therapeutic application. It is likely that there will be evolution of new regulatory frameworks to control development of new stem cell based therapies and generation of transgenic animals. The safety and efficacy of the next generation of stem cell based products must be ensured by providing an appropriate structure through the existing regulations pertaining to products (biological) as well as tissues. The field of tissue engineering must be given special attention.

Above all, it is mandatory for the clinicians, researchers and scientists to be aware of the regulations concerning stem-cell based therapies and at the same time their application to ensure safety to animal and human population. Much is needed to be understood and explored to propagate, popularize and take advantages of stem cell therapy and its other useful applications in biomedical research that include both veterinary and medical field for safeguarding health of humans and animals.

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