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Therapeutic potential of stem cells in veterinary practice

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Abstract

Stem cell research acquired great attention during last decade inspite of incredible therapeutic potential of these cells the ethical controversies exists. Stem cells have enormous uses in animal cloning, drug discovery, gene targeting, transgenic production and regenerative therapy. Stem cells are the naïve cells of body which can self-renew and differentiate into other cell types to carry out multiple functions, these properties have been utilized in therapeutic application of stem cells in human and veterinary medicine. The application of stem cells in human medicine is well established and it is commonly used for chronic and accidental injuries. In Veterinary sciences previous studies mostly focused on establishing protocols for isolation and their characterization but with advancement in array of techniques for in vitro studies, stem cells rapidly became a viable tool for regenerative therapy of chronic, debilitating and various unresponsive clinical diseases and disorders. Multipotent adult stem cells have certain advantages over embryonic stem cells like easy isolation and expansion from numerous sources, less immunogenicity and no risk of teratoma formation hence their use is preferred in therapeutics. Adult stem cells have been utilized for treatment of spinal injuries, tendonitis, cartilage defects, osteoarthritis and ligament defects, liver diseases, wounds, cardiac and bone defects in animals. The multi-potential capability of these cells can be better utilized in near future to overcome the challenges faced by the clinicians. This review will emphasize on the therapeutic utilization and success of stem cell therapies in animals.

Keywords: Stem cells, Differentiation, Characterization, Regenerative therapy

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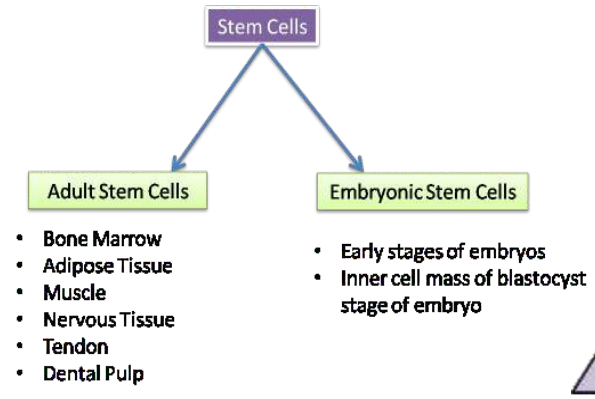
Introduction

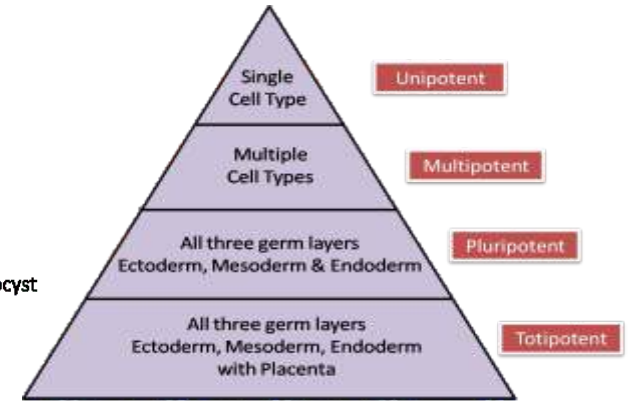
Stem cells offer an unprecedented hope in treating many debilitating diseases of humans as well as animals. They have two important distinguishing characteristics, first they are unspecialized cells capable of self-renewal through cell division, sometimes after long periods of inactivity and second, under certain physiologic or culture conditions, they can be differentiated to tissue- or organ-specific cells with special functions. Stem cells can be classified as adult and embryonic stem cells; adult stem cells are derived from adult body organs whereas embryonic stem cells from embryo. In adults, stem cells act as a repair system by replenishing tissues of the body whereas in embryonic stem cells differentiate into all the specialized cells and develop into adult.

Stem cells are valuable tools for gene targeting [1], cloning [2], chimera production [3] and transgenic animals [4] which brought much excitement in the field of stem cell research. Stem cells retain the ability to become some or all of the more than 200 different cell types in the body hence, unique regenerative potential of these cells make their use indispensible in the area of therapeutics. Stem cells are undoubtedly, most promising for cell-based therapies that are currently tested in pre-clinical trials for a wide range of ailments for their therapeutic potential. The basic mechanism of action and generalized applications of mesenchymal stem cells were reviewed by various researchers [5,6]. Most of the reports mainly focused on orthopedic injuries of horses which exist as a big challenge ahead of clinicians [7,8]. The current compilation emphasizes on the preclinical and clinical studies of the utilization of embryonic and adult stem cells in laboratory animals, pets and farm animals. The standard research databases like MEDLINE, AGORA and Google Scholar search engine on internet were used for collection of full papers and abstracts



Source: Spencer et al., 2011 [102]

Figure 1: Sources of Stem Cells



Source: Boiani et al., 2005, [103]

Figure 2: Plasticity of Stem Cells

however some papers were also collected from list of references of other papers.

Sources of stem cells

Stem cells can be classified as Embryonic Stem Cells (ESCs) and Adult Stem Cells (ASCs) depending on their source (Figure-1). The plasticity is the ability of stem cells to differentiate which may form one cell type or multiple types of cells (Figure-2). Totipotent means ability to differentiate into all three germ layers including placenta similarly pluripotent stem cells can form all three germ layers except placenta. The multipotent stem cells can differentiate into multiple cell types of particular tissue type whereas unipotent can form only one cell type [9]. The first successful report of mouse embryonic stem cells (ESCs) [10,11] was followed by isolation and characterization of ES cells in other species like hamster, mink, rabbit, rat, monkey, marmoset, chicken, human, baboon, dog, cat, horse, pig, cow, sheep, goat and buffalo [12-23]. Adult stem cells are of various types, hemopoeitic stem cells, mesenchymal stem cells, neural stem cells, skin stem cells, retinal stem cells etc. [24]. Mesenchymal Stem Cells (MSCs) can be isolated from bone marrow, fat, umbilical cord blood (UCB), amniotic fluid, placenta, dental pulp, tendons, synovial membrane and skeletal muscle [25-33]. They have the potential to differentiate into cells of various tissues like fibroblasts, muscle, bone, tendon, ligament, and adipose tissue [34,35,36,37]. Friedenstein and co-workers [25] isolated these cells as colony-forming unit fibroblasts from murine bone marrow. Further, Caplan (1991) for the first time named these cells as mesenchymal stem cells. MSCs have been isolated from a number of species like human, rat, mice, dog, cat, pig, horse, sheep, goat, cattle [11,38-46]. MSCs can be isolated and cultured easily with high *ex vivo* expansion rate. This makes these cells an attractive tool in regenerative medicine for cell therapy [45].

Stem cells in veterinary regenerative therapy

Stem cell therapy involves various routes for transplantation of cells into patients like local delivery or systemic infusion. Stem cells can be derived from same animal (autologous), same species (allogeneic) or from different species (xenogenic) for transplantation. Recent studies report many interesting examples of the therapeutic use of MSCs and ESCs [47-49]. Stem cells address a broad spectrum of indications, including spinal cord injury, bone, cartilage and cardiovascular repair. In 1968, first successful allogenic stem cell graft in humans using donor bone marrow was undertaken in the United States of America [50,51]. Currently, there are many research groups studying *in vitro* expansion of these and other stem cells for direct clinical applications [52]. Stem cells must be characterized according to criteria decided by International Society for Cell Therapy (ISCT) before they are utilization for therapy [53]. In animals, MSCs derived either from bone marrow or adipose tissues for their routine use in experimental studies and clinical cases [54,47]. MSCs are beneficial in handling various chronic and debilitating clinical conditions of canines, equines and caprines though there are very limited reports in large ruminants on therapeutic application of stem cells research is still going. Many private companies like Vetstem (U.S.A., http://www.vet-stem.com/), Medistem Inc. (U.S.A., http://medisteminc.com/), Histostem (South Korea, http://www.histostem.co.kr) provide stem cell therapy for animals and claims cure for orthopedic and other injuries (for websites see

reference). Recently, some institutions like North Carolina state university, Cambridge university were in news for treatment of canine spinal injuries.

Spinal injuries

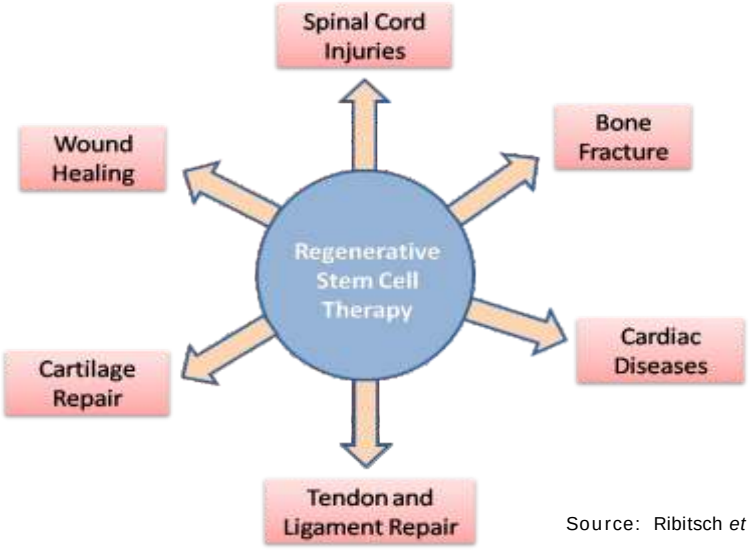
The remarkable developmental potential and replicative capacity of human embryonic stem (ES) cells were utilized by transplanting neural precursors into the brain of immunosuppressed neonatal mice and no teratomas emerged within 8 weeks after implantation [55]. In similar way neural progenitor population of cells was used in the successful treatment of a Parkinson's rat model for 12 weeks with no teratoma formation [56]. hESC derived neurons were injected in primate and rodent models for treatment of neuronal injuries without tumor formation [57]. 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 challenge to modern therapeutics. Therefore transplantation of stem cells with the ability to differentiate into neurons and supporting cells may be a practical method for recovery. The differentiation potential of stem cells was assessed and they not only they differentiated but also integrated into axonal pathways and thus aid in regeneration of injured nerves [58]. Xenogenic transplantation of human UCB stem cells in rats following spinal cord injury significantly enhanced locomotor function within 14 days after therapy as compared to the control group [58]. Bone marrow derived MSCs were first used in Rhesus monkeys for nervous tissue regeneration which appeared promising [59]. Intrathecal implantation of autologus bone marrow derived MSCs improved locomotor activity significantly in six dogs within one week [60]. Similarly allogenic UCB derived MSC transplantation resulted in nerve regeneration in canine fetuses. In UCB-MSC treated group animals the gait was improved in 2 weeks and the weight bearing power of the pelvic limbs was also improved. The improved nerve conduction velocity and distinct structural consistency of the nerve cell bodies was observed in lesions treated with MSCs [61]. Encouraging results were reported by same group in which adipose derived stem cells (ADSCs) were used to treat spinal injury in canine [62]. The comparison of autologous and allogenic transplantation of canine bone-marrow derived MSCs in experimentally-induced spinal cord injury (SCI) revealed that both approaches could be utilized clinically [63].

Cardiac defects

Laflamme and co-workers [64] injected differentiated cardiac-enriched hESC progeny into the left ventricular wall of athymic rats and found that grafts consisted predominantly of cardiomyocytes by 4 weeks. hESCs can form human myocardium in the rat heart, permitting studies of human myocardial development and physiology and supporting the feasibility of their use in myocardial repair. Mouse ES derived cardiomyocytes were engrafted in injured myocardium of rat this resulted in an angiogenetic effect, and subsequently improved cardiac function during the 32-week observation period [65]. Menard and associates [66] transplanted murine ES derived cardiomyocytes in 18 sheep in which myocardial infarction was induced they found that ESCs were colonized in the scar area and accompanied by a functional bene?t of the damaged myocardium. The findings obtained in a clinically relevant large-animal model of heart failure strengthen the potential therapeutic use of ESC to regenerate the severely dysfunctional myocardium and bring additional evidence for an immune privilege of these cells.

Cartilage defects

Mouse ES cells (AB2.2 or CCE cells) were transplanted into articular cartilage defects in the patellar groove of immunosuppressed rats and cells were observed 8 weeks after transplantation. Cells produced cartilage resulting in repair of defect this indicated environment of osteochondral defects is chondrogenic for ES cells [67], where as another group created full-thickness osteochondral defects on the patella groove of SD rats, and treated these rats with ES cells embedded in collagen gel. Thirty-five ES-like colonies from 40 IVP sheep embryos, positive for stage-specific embryonic antigens (SSEAs), were pooled in groups of two or three, embedded in fibrin glue and transplanted into osteochondral defects in the medial femoral condyles of 14 ewes [68]. Sheep ES-like cells transplanted into cartilage defects stimulate the repair process to promote better organization and tissue bulk. The utilization of MSCs for the repair of cartilaginous tissue that is difficult to heal in adult animals. MSCs can differentiate into chondrogenic lineage [69] and utilized to treat cartilage defects. The articular cartilage defects were treated with MSCs with polymers [70], type I collagen [71], and polylactic acid [72]. Infrapatellar fat pad derived mesenchymal stem cells were used in rabbits for treatment of osteoarthritis [73]. Caprine osteoarthritis model showed local delivery of adult mesenchymal stem



Source: Ribitsch *et al.*, 2010 [104]

Figure 3: Therapeutic Potential of Stem Cells

cells to injured joints stimulates regeneration of meniscal tissue and retards the progressive destruction [74]. Chronic osteoarthritis in 21 dogs was treated with autologous adipose derived MSCs and all treated animals showed improved gait [75]. Canine mesenchymal stem cells (MSCs) seeded in type I collagen-glycosaminoglycan (CG) matrices were used in 10 dogs for repair of cartilage defects of knee joints [76]. The treatment of cartilage defects is challenge to practitioners though polymer based treatment is used it does not provide efficient cure. Another major obstacle to the application of MSCs in cartilage repair is improving the integration of neocartilage matrix with the surrounding native cartilage matrix. In large-animal models, sheep were treated with in vitro differentiated MSCs for repair of chronic osteochondral [77]. Delivery of bone marrow concentrate to acute full-thickness cartilage defects has the clinical potential to improve cartilage healing in equine model [54]. MSC therapy provides a simple, arthroscopically applicable, and clinically effective approach for cartilage repair.

Tendon and ligament repair

The mechanical properties of healing tendons and ligaments are not comparable to those of normal tissue, the quality of the tendon and ligament healing can be improved with altered therapeutic strategies which include stem cell therapy. Bone marrow derived autologous MSCs along with collagen gel were used to repair surgically induced patellar tendon defect in adult New Zealand White rabbits, treated group shows significant improvement in its biomechanical properties after 4 weeks [78]. Similar combination was used for Achillies tendon repair in rabbit model [79]. It was found that MSCs treated groups better regain the normal tendon maximum force, stress, modulus, and strain energy density compared with controls. In equine, autologous bone marrow derived MSCs after in vitro expansion were utilized and found effective for regeneration tendon matrix in superficial flexor tendon injury [80]. The collagenase induced tendinitis in the superficial digital flexor tendon in 8 horses was treated with adipose derived nucleated cells (ADNC) injection. The treated group showed improvement tendon organization which was assessed by cartilage oligomeric matrix protein (COMP) expression [81]. In race horses, the adipose derived MSCs were used to successfully treat experimental tendinitis [47].

Ligament healing can be enhance by transplantation of mesenchymal stem cells (MSCs), which are demonstrated to differentiate into fibroblast-like cells in ligament injury sites in rats and survive upto 28 days [82]. Equine suspensory ligament injuries are challenging because healing process is slow and re-injuries are common. The bone marrow components were injected for recovery from ligament injuries in 100 horses and found effective [83]. Therapeutic application of adult stem cells in equine tendonopathies and orthopedics is well studied and many reports are available on their clinical use [7,8].

Bone Repair

The MSCs can undergo osteogenic differentiation, and exploration of the potential for using autologous stem cell therapy to augment bone repair and regeneration is well reported. MSCs stimulates of 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 [84,85]. The preclinical studies were carried out in laboratory animals like rat, rabbit. Canine segmental bone defects were treated with autologous bone marrow derived MSCs loaded onto porous ceramic cylinders. The results obtained were encouraging showed significantly greater amount of bone as compared to control [86]. Further, non-union fracture in dog was cured by autologous transplan-tation of adipose derived MSCs with hydroxyapatite and chitosan scaffold [87]. In another study allogenic transplantation of canine MSCs proved worthy for repair of critical sized segmental defects [88].

Among large-sized animals the use of sheep autologous BMSC in conjunction with hydroxyapatite ceramic (HAC)-based carriers results in faster bone repair compared to hydroxyapatite ceramic HAC alone [89]. Goat Bone marrow derived MSCs cultured with scaffolds could repair the segmental bone defect in tibia by 8 weeks after surgery [90]. These reports demonstrate the feasibility and efficiency of using MSCs to augment the repair of bone defects in animals.

Wound Healing

The effects of embryonic stem cells (ESCs) on diabetic wound healing were investigated using an excisional skin wound model in 110 diabetes-induced rats. The topical ESCs injections enhanced diabetic wound healing during the early stage, and suggested that ESCs offers a novel therapeutic modality for the treatment of diabetic wounds [49]. Krause and associates [91] found that adult bone marrow cells give rise to epidermal keratinocytes, follicular epithelial cells, sebaceous gland cells, dendritic cells after their transplantation in mice. Bone marrow derived mesenchymal stem cells were injected around wound and their application to the wound bed in an excisional wound model enhanced healing significantly in normal and diabetic mice [92]. Autologous bone marrow derived nucleated cells transplanted in experimental rabbits and clinical cases to evaluate their tissue regeneration potential in full thickness wounds [93], burn wounds [94] and corneal alkali burn wounds [95]. In xenogenic model human MSCs were used for incisional wound healing and tissue regeneration in rabbit and fetal sheep [96,97]. In caprine Wharton's jelly mesenchymal stem cells (WJMSCs) of umbilical cord were used to treat cutaneous wounds in goat. Results showed complete re-epithelialization at day 7 in treated group with less inflammation, thinner granulation tissue formation with minimum scar [48].

MSCs were also found useful for treating cerebral infarction [98] and ischemia [99], myocardial infarction [100], autoimmune disorders [101] in experimental models.

Conclusion

Stem cells are undoubtedly, most promising for cell-based therapies thereby provides a powerful and flexible option for veterinarians to restore function and improve animal health through the novel techniques. In veterinary sciences, stem cells are mainly used for the treatment in canines and equines whereas research is still going on in other farm animals. However their extensive use in all clinical condition cannot be recommended because of high treatment expenditure yet, certain chronic and irreparable conditions can be better treated with these cells and the cost of production will hopefully be reduced when extent of production is increased. Though stem cells have immense potential in therapeutics their clinical use requires extensive research for standardization of the treatment protocols, routes and doses. Further, guidelines and regulations for the controlled use of stem cells in animals will become a near future need. The modern vets-scientist team efforts will play a pivotal role in the development and implementation of these innovative strategies to ultimately improve livestock production and pet care.

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