Kulus et al.
Medical Journal of Cell Biology 2018
DOI: 10.2478/acb-2018-0016
Received: 22.10.2018
Accepted: 17.11.2018

The use of mesenchymal stem cells in veterinary medicine

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Abstract

Constant advances in medicine, both human and veterinary, lead to continuous discovery of new drugs and treatments. Recently, the aspect of stem cell use in regenerative medicine has been very popular. There are still too few clinical trials on animals that could precisely estimate the therapeutic efficacy of cell therapy. However, stem cells are a source of extraordinary potential for multiplication and differentiation which, if used properly, can prove to be an effective mean of treatment of numerous diseases that are currently considered untreatable. The purpose of review is the characterization and clinical use of stem cells in mostly occurring diseases. Particular attention has been given to the issue of mesenchymal stromal cells, which so far have been most widely used in clinical practice. Current research into stem cells has allowed scientists to discover many different types of these cells, describe their characteristics and divide them into groups, with the most important being embryonic stem cells and somatic (adult) stem cells. Adult stem cells, due to their availability and lack of ethical problems, are used in veterinary practice. Different types of mesenchymal stem cells are distinguished, based on their origin. Adipose tissue derived stem cells and stromal vascular fraction find the widest clinical application. In veterinary medicine, stem cells therapies are most commonly used in the case of horse orthopedic injuries and in diseases of various origin in dogs and cats. While further research is needed to confirm the effectiveness of cell therapies, they have much potential to find plenty of potential applications in future medicine.

Running title: The use of stem cells in veterinary practice

Keywords: mesenchymal stem cells, regenerative medicine, veterinary, equine stem cells, feline stem cells, canine stem cells

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Introduction

The multitude of therapeutic substances used nowadays allows to treat many diseases. However, medicine is not always able to overcome deadly illnesses. Stem cell based regenerative medicine offers opportunities to improve the health of many people and animals who were condemned for poor prognosis. The beginnings of this method can be tracked to discovery of ways to derive embryonic stem cells form mouse embryos in 1981. This led to development of methods to derive stem cells from human embryos and grow these cells in the laboratory, in 1998. Thomson et al. [1] in 1998 isolated embryonic stem cells (ESC) from human blastocyst, but until now, only mouse ESC have been investigated in depth [2]. Fortier et al., in 1998, isolated mesenchymal stem cells from adult horses and determined the condition of their in vitro culture [3]. In 2001, mesenchymal stem cells were identified in adipose tissue, which had a significant influence on further research and lead to easier access to adult stem cells, which can be used in practice [4]. For the first time, dog SVF were obtained in 2001, when the inguinal fat extracted cells were cultured in the laboratory. Methods of their isolation and cultivation in primary culture were also described [5]. Another breakthrough took place in 2006, when the conditions which allowed some specialized adult cells to be reprogrammed genetically to achieve stem cell like state were discovered. These cells were named induced pluripotent stem cells (iPSCs).

Stem cells have a great potential to develop into many different cell types in organisms. They can divide without limits in many tissues, to replace old or defective cells, if the organism is still alive. Therefore, they are identified as internal repair system. Each new cell originated from stem cell can remain a stem cell (save the potential to divide) or become another type of cell (which is more specialized). Stem cells are unspecialized cells and they can renew themselves through division. In addition, they can become specialized cells of organ or tissue when induced. The gut and bone marrow are prominent stem cell rich organs, where the constant stem activity is required due to constant need for cell production. Other organs such as heart or pancreas, also possess stem cells. However, they only divide when particular conditions are met. Adult stem cells can be found in developed organisms, but they can only differentiate into cell types proprietary to tissues in which they reside [6].

Although cellular therapies have been commercially used in veterinary practice for several years, little is known about the real effectiveness of this method. The biology of stem cells, the conditions necessary for their development and the mechanisms of their differentiation are very interesting issues for scientists all over the world. Therefore, a large number of scientific institutions are devoted to the research of these cells, which results in a huge number of publications in this area. Stem cells are important resource and promise for cell – based therapies, disease modeling, drug discovery and many others [7]. Stem cells are already used in laboratory to screen new drugs [8]. However, the available literature mostly describes studies of laboratory induced malignancies. So far, only a small number of studies have been carried out on animals naturally affected by the diseases. Therefore, further systematic and controlled research, carried out on live animals with natural pathology is still needed. The purpose of this study is to review the characterization and clinical use of stem cells in mostly occurring diseases in veterinary practice. Particular attention has been given to the issue of mesenchymal stromal cells, which so far have been most widely used in practice.

Stem cells types

Scientists distinguish two groups of stem cells, based on their origin: embryonic stem cells (ESC) and adult (somatic) stem cells (ASC).

Embryonic stem cells are undifferentiated cells that are derived from preimplantation-stage embryos. They can divide without differentiating for a prolonged period in culture and become any cell type. The wide differentiation potential of ESCs makes it possible that they will play an important role in a regenerative medicine in future. So far, ethical problems and the risk of cancer due to uncontrolled divisions have left ESC research in the pre-clinical phase [9].

Adult stem cells are undifferentiated cells found in many organs and adult tissues, with a limited capacity for both self-renewal (in the laboratory) and differentiation. ASCs have different differentiation abilities, most often limited to cell types proprietary to the organ from which they originate. Adult stem cells, which are present in in almost all tissues of a mature organism, are a regenerative reserve. They are even located in the nervous tissue, which has poor regenerative capacity [10]. The adult stem cells population is very small. Hence, isolation can be difficult and the potential for self-renewal and differentiation is much lower than that of ESCs. They are usually multipotent or unipotent. However, there are no ethical problems with ASCs, which is important in human medicine, as it is possible to use ASCs for autologous transplantation (the safest for patients) [9].

Mesenchymal stem cells (MSC) are non-blood, adult stem cells, present in a variety of tissues. MSCs exhibit the plastic adherence properties and form spindle-shaped colonies. These cells develop from mesoderm and present multipotent properties [2]. They are present in all tissues in body, primarily functioning during organ renewal [11]. MSCs are self-renewing, easily accessible and expandable

in vitro, with exceptional genomic stability. With MSCs, there are no major ethical constraints, so they are an important potential cell source for cell therapy, tissue repair and regenerative medicine [2]. MSCs are stimulated by growth factors and hormones. They acquire morphophysiological aspects pertinent to their localization in the organism. MSCs are used for treatment of tendon and ligament, injuries, as well as joint diseases. They can be isolated from bone marrow or adipose tissue through minimal manipulation. In horses and dogs this kind of treatment has significant clinical relevance [11]. MSCs from diverse sources exhibit large differences in the expression of proteins important for cell viability and migration. MSCs have the potential to differentiate into various cells, including hepatocytes and neurons [12].

The healing abilities of MSCs most probably result from their strong properties to secrete many types of molecules, which influence the immune system, exhibit anti-apoptotic, anti-inflammatory, chemotactic or proangiogenic functions, as well as stimulate tissue regeneration. Since MSCs are present in all tissues and are responsible for regeneration, the treatment with MSCs should focus on activation of as many endogenous MSCs as possible in patients. However, in older patients with advanced lesions, severely injured, endogenous supply of MSCs may not be sufficient. In such patients it would be advisable to use exogenous stem cells for the treatment. In turn, during exogenous stem cell therapy many of the cells do not reach the trauma site, which causes minimal therapeutic benefits, as indicated by clinical trials [12].

Recently, there have been voices to change the name of MSC's. The current name (which was created more than 25 years ago) does not reflect the true nature of these cells, while also misleading patients using MSCs. These cells do not constitute to the real cell reserve, but only by their strong secretional and biomodulatory action they stimulate these reserves in the body. They operate in injury areas, so that regeneration mechanisms can be activated. The name MSC should embellish the true mechanism of their operation. The author proposes the name "Medical Signalling Cells" – which in his opinion better illustrates the mode of their therapeutic action [13].

In practice, MSCs obtained from adipose tissue are often used. One of the cell fraction types collected by this method is stromal vascular fraction (SVF). This fraction contains several cell types that are present in the extracellular matrix of adipose tissue. In laboratory, the adipose tissue is incubated with enzymes, which leads to release of cells from matrix. Then, the suspension is centrifuged. The population of adipocytes floats on top of the sample, with SVF contained in the pellet at the bottom of the tube [14]. The SVF is also used to harvest adipose-derived stem cells in cultures. In order to achieve that, the cells are incubated at 37˚C and 5% CO_2. Non-adherent cells are removed, leaving only adherent ADSCs in the culture.

Many animal diseases could be treated with stem cells. These cells have high therapeutic potential and can be introduced as autologous or allogenic, freshly isolated or from in vitro culture. Many animals have already been treated with stem cell therapy. Therefore, it is possible to determine the effectiveness of its effectiveness. In human, on the other hand, only the treatment of hematological diseases, in which bone marrow transplantation is used, is permitted. Despite the wide use of stem cells, their exact therapeutic mechanism has not been fully understood. Today, MSCs from various sources, mainly form adipose tissue and bone marrow, are used worldwide to treat animal diseases [11].

Mechanisms of action

Stem cells have three characteristic properties: they can divide and renew themselves, are unspecialized, and can specialize into another type of cell. The principle of stem cells function is to send and receive signals within their specific niche. These signals are autocrine, paracrine, endocrine and intracellular. When they are sent, the mechanisms of cell proliferation and differentiation are activated. Stem cells have specific receptors on their surface which respond to appropriate ligands. The more ligands are sent, the larger the gradient is created and more receptors are activated [11].

A process called *cell homing* is an essential mechanism of cell therapy. It involves activation of a specific cellular signaling axis and its response, which affects the colonization of cells at their destination [11][15]. It is well known that chemokine SDF-1 (stromal cell derived factor 1, also known as CXCL12), plays a very important role in this process, which is associated with a specific receptor (CXCR4) on the surface of the cell membrane of the stem cell [12]. The data provides evidence that SDF-1 controls the migration and reproduction of mice primary cells, controls the setting of regenerating primary cells in the bone marrow and influences the liberation of mature cells into peripheral blood. These processes are additionally supported and controlled by many different factors (e. g. adhesion molecules) [15].

SDF-1 is a chemokine which is produced by stromal cells in bone marrow, as well as by epithelial cells in various organs. The expression of this chemokine was observed in pancreas, spleen, ovaries, small intestine, and in smaller amount in brain. There was no evidence for SDF-1 production by peripheral leukocytes [15]. In patients with diagnosed ovarian cancer, increased SDF-1 secretion has been demonstrated. The high concentration of this chemokine induces angiogenesis, even if the VEGF concentration is low [16]. Chemokine receptors are

built form seven characteristic loops, penetrating cellular membranes, with their intra-cytoplasmatic part being a G-coupled protein. The CXCR4 receptor is not the only receptor for SDF-1. This chemokine have two versions (SDF-1α and SDF-1β), and both of them can connect with two types of receptors: CXCR4 and CXCR7 [15].

It has been proven that human MSCs are capable of secreting anti-inflammatory and antibacterial factors [17]. Researchers have shown that MSCs decrease inflammation and *Pseudomonas aeruginosa* inception in the *in vivo* murine model of cystic fibrosis (CF) [18]. Another feature of MSCs is their immunomodulating effect on immune cells such as T and B lymphocytes, dendritic cells, NK cells and monocytes. ASCs had a greater potential to differentiate towards osteoblasts than bone marrow MSCs [14].

Isolation and culture of adipose – derived stem cells

Human stem cells from adipose tissue are obtained from elective liposuction procedures under local anesthesia [4]. In the case of animals, fragments of adipose tissue are collected during surgical procedures, under general anesthesia, from the inguinal region in dogs and cats, and from the tail base in horses [14]. The raw lipoaspirate is washed with phosphate – buffered saline (PBS), and t digested at 37˚C for 30 min, using collagenase. Afterwards, the enzyme's activity is neutralized with Dulbecco's modifies Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). Then centrifugation is then carried out at 400 x g for 10 min, to obtain SVF pellet [19]. The pellet is resuspended in NH_4Cl and incubated at room temperature for 10 min to lyse contaminating red blood cells [4]. The centrifugation is then repeated, with SVF filtered through nylon mesh and incubated at 37˚C and 5%CO_2 in culture medium. After overnight incubation, the plates are washed with PBS to remove nonadherent and obtain a homogenous culture of ASCs [14,4].

Stem cell use in veterinary practice

The treatment with stem cells is most often used in orthopedic patients in whom pharmaceutical treatment is no longer effective (i.e. hip dysplasia in dogs) [19]. In veterinary medicine, stem cells isolated from adipose tissue (ASCs) have a big therapeutic potential. ASCs are easy to collect, exhibit large abundance and regenerative abilities. In 2013, the International Federation of Adipose Therapeutics and International Society for Cellular Therapy proposed minimal criteria to define SVF and ASCs as fresh stromal vascular fraction and cultivated ASCs [20]. The human MSCs also have their general identification criteria established: 1 – capacity to proliferate as adherent cells in cultures, 2 – ability of in vitro differentiation (into osteoblasts, adipocytes, chondroblasts), 3 – phenotypically positive for CD73, CD90, CD105 and negative for CD14, CD11b, CD34, CD45, CD19 or CD79α. Immunophenotyping of animal stem cells is more limited because of lack of many antibodies necessary for the definition of the surface profile [14].

In veterinary practice ASCs are mainly used for treatment orthopedic pathologies: ligament, bone, tendon and joint injuries. The MSCs are usually obtained from bone marrow, umbilical cord, adipose tissue and Wharton's jelly [19]. Stem cells used in clinic therapy should be easy to obtain, requiring minimal manipulation, which has a low risk of infection of the material. Stem cells may be administrated as an autologous transplant or, after collection from a donor and cultivation in laboratory, as allogeneic material.

Equine stem cells

The rough use of stem cells is found among the horses used in sport. The abundance of orthopedic injuries leads to the search for new methods of treatment, because of sports horses' usual value and the need to protect them for factors that might cause early retirement. As surgical procedures are very invasive and require long recovery times, the use of stem cell therapy seems to be a future for equine medicine. Fortier et al. described mesenchymal stem cells derived from bone marrow of two adult horses [3]. This study shown that MSCs from adult horses have the capacity to undergo chondrogenic differentiation in monolayer cultures. Authors suggested that equine MSCs from bone marrow may be the source for autologous transplantation in articular cartilage repair.

Most frequently injured tendon in horses is the superficial digital flexor tendon (SDFT), especially prominent in the forelimb of jumping horses. Dressage horses are often affected with hind limb suspensory ligament (SL) injury [21]. Twelve horses with naturally occurring SDFT injury were used in research on the use of bone marrow derived MSCs [22]. The treated group received BM-MSCs injections into damaged tendon, with the control group only injected with saline. Tendons were examined 6 months after injection, when the horses were euthanized. The result showed a significant improvement of the healed tendons, less stiffness and a better histological organization in the stem cell treated horses, in comparison to the control group. The authors also suggested the possibility of using such therapy in the treatment of tendon defects in human [21]. Further tests were performed, using allogeneic MSCs: form umbilical cord blood in 52 horses [23], and adipose tissue in combination with platelet rich plasma in 19 horses [24]. In both cases positive results were obtained in horses with tendon trauma. A study comparing the use of MSCs and PRP was also conducted, as well as a combination of PRP and MSCs in the treatment of tendon trauma in horses.

It turns out that the combination of PRP and MSCs gives much better results than using these methods separately [25]. The use of autologous MSCs is associated with a longer period of time from diagnosis to treatment. More precisely, a 2-3 week culture period is required before implantation in the lesion, which may have negative effects on treatment [21].

Equine tendonitis therapy, using mesenchymal stem cells and platelet concentrates was examined in the group of 8 horses with experimentally induced lesion of SDFT [26]. Researchers have used the adipose derived mesenchymal stem cells suspended in platelet concentrate with phosphate buffered saline, which were administrated into the lesions. The treatment brought the expected results. Histopathological studies shown no progress of lesions, increased organization and reduced inflammation.

Beerts et al. [21] conducted a study with the use of tenogenically induced mesenchymal stem cells and platelet – rich plasma in SL and SDFT lesions. In this research 68 horses with SL lesions and 36 horses with SDFT lesions were used. Allogeneic tenogenically induced MSCs with PRP were injected into the lesions. The control tests were carried out after 6 and 12 weeks and after 12 and 24 months. The lameness and ultrasound tendon structure were studied. In coclusion, positive results have been achieved, as the lameness was largely abolished, and the ultrasound showed significant improvement.

A recently published study revealed, for the first time, a successful attempt to obtain MSCs from the horse's uterus [27]. Researchers demonstrated the presence, in the endometrium, of cells that fit the definition of mesenchymal stem cells. This discovery may point to another source of MSCs, which is easily accessible and not invasive in the method of harvesting. The therapy with these cells would not have to concern only the uterus but various tissues, because these cells show differentiation towards adipogenic, chondrogenic, osteogenic and smooth muscle lineages.

Feline stem cells

Quimby et al. [28] investigated stem cell therapy in cats with chronic kidney disease (CDK). The autologous intrarenal mesenchymal stem cells (MSCs) was used in six cats. Two of them were healthy and four exhibited CKD. All animals received a single intrarenal injection of bone marrow derived stem cells or adipose tissue derived stem cells, via ultrasound guidance. Glomerular filtration rate (GFR) and other database were determined before injection and after 7 and 30 days post-injection. Modest improvement in GFR and mild decrease in serum creatinine concentration was observed in two of cats with CDK (injection of aMSCs). However, researchers argue that broad clinical use is unlikely, due to high invasiveness of the administration method (sedation and intrarenal injections). They recommend looking for alternative methods.

Other research on feline stem cells has been carried out to compare bone marrow MSCs and adipose tissue MSCs, with respect to their in vitro growth and cell surface phenotype [29]. It has been observed that adipose derived stem cells proliferated significantly faster. Cultures of adipose derived stem cells were described as easier to generate because of their proliferation potential. ASCs exhibited normal mesenchymal type morphology and trilineage differentiation potential. Immunophenotypic differences were not present. Cells from both groups were positive for CD44, CD90, CD105, and negative for CD4 and MHC class II. Larger antibody panel was described in another study of feline MSCs [30]. ASC immunophenotyping showed that the cells were also positive for alpha smooth muscle actin and negative in for CD14, CD34, CD45. In addition, the cells demonstrated the ability to differentiate towards adipogenic, osteogenic, chondrogenic and smooth muscle cell lineages in vitro.

Canine stem cells

In dogs, hip dysplasia (HD) is a common pathology, which occurs in all breeds with different prevalence. Clinical symptoms are pain and lameness. Treatment can be conservative (administration of analgesics or chondroprotective drugs) or surgical. These treatments can be poorly effective or be very invasive for patients. Marx et al. showed a clear improvement after week of treatment of all dogs (n=4) with autologous SVF therapy, and in three (n=5) dogs treated with allogenic ASCs [19]. They used adipose tissue from the inguinal region of the dogs. Therefore, they recommended hip dysplasia treatment with autologous SVF or allogenic ASCs by acupoint injection. Black et. al described utilization of autologous SVF in treatment of chronic osteoarthritis (OA) of a coxofemoral [31] and humeroradial [32] joints in dogs, with good results. They described that dogs that underwent treatment showed lower lameness and pain and had better range of motion, compared to control dogs.

Researchers have also shown that acupoint injection of stem cells improved blood circulation in the case of hind limb ischemia in rats [33]. Bone marrow mesenchymal stem cells were used. The condition was induced experimentally, by blocking the femoral artery and its branches. Three weeks after injection two muscles were taken from the ischemic side. With immunohistochemical methods, muscle samples were examined to determine the expression of vascular endothelial growth factor (VEGF) and transfer growth factor-β_1 (TGF- β_1) – which have a significant role in creating new blood vessels. The density of small arterial vessels in these muscles was also examined with immunohistochemical methods. The serum levels of VEGF and nitric oxide (NO) were investigated. The study utilized 24 laboratory rats, including control group. These

studies have shown that angiogenesis and arteriogenesis has significantly increased, indicating by an increase in TGF- β_1, VEGF and NO.

Advancing age of the donor has been described to have a negative effect on the frequency and function of MSCs. On the other hand, autologous transplants have proven far more successful in clinical trials [19]. It has been evaluated that canine ASCs cultivated with reduced level of oxygen have smaller potential for proliferation and differentiation [34]. This is a very important information for clinical treatment in environment of hypoxia (such as fracture site). A crucial aspect concerning the clinical use of ASCs is the transport of material. It has been proven that transport time and temperature influence the number, gene expression and acquired resistance to apoptosis of ASCs in cultures. It was found that canine ASCs should be delivered in a PBS solution, at room temperature, during 9-12 hours [14].

Canine ASCs have also found use as donor cells for somatic cell nuclear transfer [35]. In addition, cloning of beagle dogs through nuclear ASC transfer was also performed [36]. Iravani et al. examined therapeutic possibilities of bone marrow MSCs and conditioned media in the treatment of laryngotracheal stenosis (LTS) [37]. In this study, seven dogs with mechanical induced trauma to laryngeal mucosa were used. BM-MSCs or conditioned media were injected in the right side of the vocal folds tissue, the left side was treated as control. Six weeks after treatment histological examination was made. Submucosa of vocal folds (both sides) noted a complete epithelialization and minimal inflammation. However, more noticeable therapeutic effects were observed on the side of BM-MSCs and conditioned media administration. The authors suggest that, due to the results obtained and low thickness of fibrosis (in comparison to control), the methods used could be a good route of LTS treatment.

Conclusion and perspectives

More and more centers around the world are starting to introduce stem cell therapy as a commercial method. It seems that use of stem cells can create practically unlimited possibilities in both human and veterinary medicine. This therapy can open many possibilities, not only in regenerative medicine but also in transplantology. The authors hope that in the perspective of several years or so the treatment of the damage after extensive burns or pancreatic transplantation with the use of stem cells will not be a problem. This is the beginning of a forward – looking path and every new discovery brings us closer to our expected goal.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest statement

The authors declare they have no conflict of interest.

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