

STEM CELLS IN VETERINARY MEDICINE – FROM BIOLOGY TO CLINIC

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Abstract: Mesenchymal stem cells are adult stem cells found in different adult tissues such as adipose tissue, bone marrow, and also in umbilical blood. Mesenchymal stem cells are capable of differentiation into bone, cartilage and adipose tissue, and several reports also suggest that mesenchymal stem cells might be capable of transdifferentiation into muscle and neural cells. In addition to differentiation potential, mesenchymal stem cells might have other beneficial properties for pathological processes and studies in recent years suggest that mesenchymal stem cells have immunomodulatory, antiinflammatory and trophic actions, contributing to the healing processes in injured/ diseased tissues. Osteoarthritis is a chronic, progressive disorder with debilitating effects in both animals and humans. It is particularly common in some dog breeds, but also fairly common in humans. Currently, there is no cure for such conditions, but studies in recent years in both human and veterinary medicine suggest that mesenchymal stem cells might have beneficial effect on chronic osteoarthritis. In our laboratory, we have developed a novel method of treating osteoarthritis using autologous adipose tissue derived mesenchymal stem cells in dogs and horses. Stem cells are collected from patients' adipose tissue, prepared in the laboratory and injected directly into affected joint(s). To prove the efficacy of this method, we have performed blind placebo study in dogs with bilateral osteoarthritis in knees, by treating one knee with stem cells and other knee with placebo (buffer used for cell delivery). Results of clinical examination revealed beneficial effect of stem cell treatment in osteoarthritic knees and x-ray imaging, and although with some limitations, results do suggest that degenerative processes in the knees treated with stem cells were limited or even reversed by the application of stem cells. Stem cells hold a great promise for the future of regenerative medicine, both veterinary and human, however, many questions about their use, potential and efficacy remain open and these will have to be studied and answered in the future years.

Key words: dog; horse; stem cells; veterinary medicine

Stem cells are special cells that have unique properties to divide indefinitely and are able to trans-differentiate into different tissues. Stem cells started to attract a lot of attention due to their possibility for use in regenerative

medicine, both in animals and humans. Stem cells are usually divided into three groups:

- Embryonic stem cells, derived from early embryos that are capable of differentiating into majority of tissues

- Adult stem cells that are present as resident cells in many adult tissues such as muscle, adipose, brain and many others and have limited potential for trans-differentiation

- Induced pluripotent stem cells (IPS cells), which are differentiated adult cells that are transformed into stem cells by genetic manipulations

Stem cells attract a lot of attention with the ideas to use these cells in regenerative medicine. In that respect, each of the three types of stem cells (embryonic, adult and IPS cells) have their own advantages and disadvantages, and there is still no clear answer which stem cells are the most suited for clinical use (1-3).

Embryonic stem cells are the cells with the higher differentiation potential. They could develop into any tissue or organ in the body and would, at a first glance, seem the best cells for use in regenerative medicine, as one cell could be used to treat many different disorders. However, embryonic stem cells have major disadvantages. One major disadvantage is collection of these cells. Cells can be only harvested from very young embryos (blastocysts) and in human medicine, this presents a large ethical dilemma whether working with such cells is ethical or not. Clearly, the ethical dilemma is not so important in the veterinary medicine although embryonic stem cells still have several disadvantages. Even if collection of embryonic stem cells in animals is not ethically questionable as with human stem cells, collection and culturing of these cells is challenging.

Furthermore, several studies have demonstrated that embryonic stem cells could form tumors *in vivo*, raising the question of safety of embryonic stem cells. Another important question in using embryonic stem cells is the potential immune response to such treatment. It would be certainly unpractical and too expensive (definitely in veterinary medicine) to prepare embryonic stem cells for individual patients (although there are ideas that in humans, cloned human embryos would be produced from patients needing stem cell treatment, and these cloned embryos could be used as a source of embryonic stem cells). Therefore, at least in veterinary medicine, embryonic stem cells from donors would have

to be used and this does raise a question of rejection of foreign cells by the patient's bodies. Although some studies suggest that embryonic stem cells are immunoprivileged and do not elicit immune response in the recipient's organism, this is far from being clear and thus, safety concerns about immune reaction to transplanted stem cells remains.

IPS cells are derived from adult differentiated cells by genetic modifications. Four genes usually have to be activated to dedifferentiate adult cells into IPS cells and these are Oct4, Sox2, c-Myc and Klf4. Although this method of producing stem cells seems very promising, easy and would overcome the problem of immune rejection of cells as cells from patients could be transformed into IPS cells, there is an important concern about the safety of these cells. Namely, several of the genes need to be activated are actually oncogenes, and IPS cells do form teratomas when injected into mice. Therefore, there are a lot of ongoing studies trying to replace activation of aforementioned 4 genes with other methods such as temporal activation, transfection of proteins instead of activating genes, use of micro RNAs and others.

The third type of stem cells is adult stem cells. Adult stem cells are present in many organs/tissues and are thought to present a reservoir of cells that can be used in the event of tissue damage. For example, myoblasts, undifferentiated muscle cells, could be activated and develop into adult muscle cells after the damage to the muscles, and similarly, neural stem cells, found mostly in the hippocampus, along the ventricles and in subventricular zone, could be activated to produce new brain cells after damage in the central nervous tissue. Adult stem cells are very interesting for clinical medicine, both human and veterinary, as they are easy to obtain, they can be obtained from the patients and thus used for autologous treatments without any danger for tissue rejection. Studies in recent years have shown that adult stem cells do not have the potential to develop into tumors such as embryonic stem cells and IPS cells. However, on the other hand, adult stem cells have much more limited trans-differentiation

potential than embryonic stem cells. It is generally thought that adult stem cells could develop into the tissues of the same embryonic layer from which they arose initially. According to this theory; ectodermal cells would develop only into other ectodermal tissues, mesodermal cells to mesodermal tissues and endodermal cells into endodermal tissues. This is not completely confirmed yet, and several studies do suggest that cells might be able to cross this embryonic layer barrier.

Two types of adult stem cells that are mostly used in development of regenerative medicine in both human and veterinary medicine today are cells from bone marrow (hematopoietic cells) and adipose tissue derived stem cells. Both are classified as mesenchymal stem cells or MSC. In particular, adipose derived MSC are very promising in regenerative medicine as they are easy to obtain in large number from adipose tissue. Although characterization of these cells is somewhat difficult, as currently we do not know any really specific biological markers for these cells, there are currently three separate lines of experiments confirming that cells obtained from the adipose tissue are really MSC. These are multiple divisions of cells in cell culture, trans-differentiation into bone, cartilage or adipose tissue and expression of certain cell surface markers such as CD 29, CD 44, CD105, CD 73 and Oct 4, although expression of these markers is not completely specific only for MSC (4, 5). Adult MSC shows a lot of potential to be used for regenerative medicine, either for producing damaged tissues *in vitro* and then using this tissues for transplantation into patients, or using cells as a treatment. Many studies in recent years are suggesting that stem cells have the capability to promote regenerative healing processes in damaged/diseased tissues. Although in many cases, such studies are still in their early phases, there is quite a lot of evidence already present that cells could indeed promote the endogenous healing processes. Studies in laboratory mice and rats, veterinary patients and also in human patients have shown beneficial effects of adult stem cells, derived from different sources in orthopedics, cardiac problems (in particularly in humans with

cardiac insufficiency, neurological trauma and even conditions like asthma. It is not yet clear what are the beneficial mechanisms through which adult stem cells induce healing, although accumulating evidence suggest that stem cells do not differentiate into damaged tissue but rather stimulate regenerative process, most likely by secreting certain, yet unknown, immunomodulatory factors and growth factors that modulate immune response /inflammation and promote endogenous healing processes.

Stem cells are attracting a lot of attention in the field of regenerative medicine. Although still in the early phases of development, stem cell treatments do show great promise for the future in both veterinary and human medicine. One of the areas where stem cells are already used is orthopedics, in both human and veterinary patients. One of the most common indications for stem cell treatments is osteoarthritis, a common disease in dogs that affects dogs of all ages. The incidence of osteoarthritis in general population of dogs is about 20 - 30 %, while in aging (older than 8 years) dogs, it can reach up to 80 %. Currently, osteoarthritis cannot be cured and dog owners are usually offered pain management. With proper pain management the quality of life of dogs is improved, although the disease cannot be cured and regular pain management therapy is both financial and life-style burden for dogs and their owners, and could have significant side effects (6). Several studies have suggested beneficial effect of adult mesenchymal stem cells in the treatment of osteoarthritis in different species from laboratory animals to dogs and horses (7-11), although there is still need for more clinical trials to confirm the benefit of MSC treatments in dog osteoarthritis.

We have performed a clinical trial in dogs with bilateral knee osteoarthritis (12). Ten privately owned dogs were included in the study with the consent of the owners. Dogs have received 2 – 3 millions of MSC into one knee and placebo (Phosphate buffer saline) in the other knee. Dogs were closely followed for one year. After one year, clinical improvement in limping was noted in 9 out of 10 dogs in stem cell treated knees. Interestingly, x-ray imaging did not reveal improvement in size of

osteophytes one year after treatment. However, while the disease progressed in all 10 joints receiving placebo, in 7 out of 10 joints treated with stem cells the radiographic score remained the same suggesting that stem cells did stall the progress of the osteoarthritis. This difference was statistically significant ($p < 0,001$).

Our results, together with other similar clinical trials do show beneficial effect of stem cell treatment in dog osteoarthritis. However, it is not yet known, how these beneficial effects are achieved. Several studies, mostly in rodents, have tracked MSC after injection into diseased joints but most studies could not find evidence of cells engrafting into the cartilage or bone tissue (13-17), some engrafting cells are detected only in synovial membranes and menisci in rabbits and goats (13, 16). However, it has been shown that in rabbits, joints treated with MSC had reduced expression of TNF-alpha, an inflammatory cytokine, and reduced expression of matrix metalloproteinase 1 (13). Similarly, studies in mice (18), goats (16) and horses (19) have shown anti-inflammatory effects of MSC in osteoarthritic joints. Therefore, it is currently thought that MSC affect in osteoarthritis might be more due to the anti-inflammatory effects, rather than regenerative capabilities. This is supported by our study as significant clinical improvement was observed in dogs on average 3 months after the treatment, but this was not accompanied by the improvement in the structure of the joints at the same time.

Beneficial effect of MSC treatment was prolonged and was evident by clinical examination at least a year after the treatment, and even more interestingly, the improvement of the cartilage appeared in some dogs 18 months after the treatment. We were not able to trace the cells in the dogs, as all dogs were clinical patients and not laboratory dogs, and therefore we could not use labeled cells for treatment. Therefore, we do not know if there was an engraftment of MSC in our study, although prolonged effect of MSC does suggest that stem cells must have engrafted into some tissues. However, this does not mean that stem cells had to engraft into the cartilage and differentiate into cartilage tissue, perhaps, as

suggested by previous studies, MSC engraft only in soft tissues and secrete anti-inflammatory and trophic factors that contribute to the healing of the joint.

In addition to osteoarthritis, stem cells, in particular mesenchymal stem cells are used in many other clinical trials/experimental treatments. Some of most promising areas are wound healing, neural trauma, cardiac and skeletal muscle disease/injuries, treatments of asthma and others. Although none of these treatments have been proven in large clinical trials, many smaller studies in both animals and humans have shown beneficial effects in various conditions. Stem cells have been shown to promote healing after myocardial infarction and tear of different muscles, in particular the semitendinosus muscle in dogs. MSC are also being studied in nerve damage, both in the spinal cord and in peripheral nerves, with very promising results using autologous porcine skin-derived mesenchymal stem cells (20). Furthermore, clinical trials are currently performed with corneal ulcers (21) and several studies, although none yet in veterinary patients, suggest an important beneficial effects in asthma (22).

Stem cells use in clinical veterinary medicine is very novel field and therefore, it is not surprising that evidence about beneficial effects are still weak. However, more and more studies, published in peer-reviewed journals do show important health benefits of stem cells in different conditions. At the moment, stem cells are most often used in treatments of osteoarthritis and tendon pathology in both dogs and horses, but large number of preclinical studies and clinical trials currently underway suggest, that the plethora of diseases treated with stem cells will most likely expand majorly in the near future.

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