THE USE OF ALLOGENEIC ADIPOSE DERIVED MESENCHYMAL STEM CELLS IN SMALL ANIMAL PRACTICE

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Animal tissue is made up of many different specialised cell types such as neurones, chondrocytes, hepatocytes and epithelial cells. All specialised cells arise from a basic stem cell.

The process of specialisation is termed differentiation. Once a stem cell has started to differentiate into a certain cell type it can no longer become any other cell type.

Stem cell sites

Stem cells are found in the embryo (blastocysts), foetus, amnionic fluid, placenta and umbilical cord blood and tissue.

After birth stem cells are found in most parts of the body including fat, bone marrow, skin, blood, lung and muscle.

Adult stem cell numbers reduce with age.

Stem cell potential.

Pluripotent cells may differentiate into any cell type. Embryonic stem cells are pluripotent, and because of this they also have the potential to form cancerous or pre-cancerous cells. Embryonic stem cells studies have largely been replaced with adult mesenchymal stem cell (MSC) studies.

Induced pluripotent stem cells are a type of pluripotent stem cell artificially derived from a non-pluripotent cell - typically an adult somatic cell - by inducing a "forced" expression of specific genes.

Multipotent stem cells are adult stem cells which can only become certain types of cells. For example haemopoietic stem cells may differentiate into red and white blood cells but not nervous tissue.

Mesenchymal stem cells are multipotent stem cells that can differentiate into a variety of cell types including osteoblasts, chondrocytes, and adipocytes. They are found in bone marrow, adipose tissue and the placenta. MSCs do not differentiate into haemopoietic tissue. Chemical factors are known to be involved in the stimulation of MSCs to undergo differentiation and form various tissues, e.g. heart muscle, pulmonary epithelial cells and joint cartilage.

The yield of MSCs from adipose tissue is much greater than from bone marrow. MSCs derived from adipose tissue are termed adipose derived mesenchymal stem cells (ADMSCs).

Mesenchymal stem cells can also be derived from the umbilical cord. The placental cord tissue (Wharton's jelly) is a rich source of MSCs. The cord blood also is a source of MSCs and stem cells derived from the cord blood are haemopoietic.

Platelet-rich plasma (PRP) is blood plasma that has been enriched with platelets. PRP contains (and releases through degranulation) several different growth factors and other cytokines that stimulate healing of bone and soft tissue. PRP is used in the treatment of osteoarthritis. Its efficacy is still to be fully validated [3].

Stem Cell properties

1. They are unspecialised cells
2. They can differentiate into a variety of other cell types
3. They can self renew
4. Stimulate healing, repair and growth of tissue.
5. Once body growth is complete stem cells are involved in the repair and regeneration of aging and damaged tissue
6. They have a paracrine role, activating host cells to secrete cytokines. Current research indicates that the release of cytokines is more important than the ability to differentiate.
7. They have very low antigenicity
8. Anti-inflammatory effect
9. Immunosuppressing properties
10. Different lines of stem cells may have different properties

Autologous and Allogeneic

Autologous MSCs are derived from the patient being treated. Allogeneic MSCs are derived from another donor patient.

Success of Autologous MSCs

There are very few scientific papers on the use of MSCs in small animals.

Black et al [1 2] has written 2 papers on the treatment of elbow and hip OA using autologous MSCs and regenerative cells. The term regenerative cells is used because the preparation of autologous cells involves the surgical removal of 20 -30 grams of adipose tissue which is then minced, washed, digested with collagenase and centrifuged to obtain the Stromal Vascular Fraction (SVF). The SVF is then resuspended in saline ready to be used. The SVF contains a variety of cells including fibroblasts, pericytes, endothelial cells, circulating blood cells and ADMSCs.

The hip study is a multicentre study showing that one IA injection of autologous MSCs reduced pain and lameness in dogs compared to a blinded saline injected control group. 18 dogs completed the study.

The elbow study is a multicentre study showing that one IA injection of autologous MSCs reduced pain and lameness. The study involved 14 dogs.

Autologous AD-MSCs have been successfully used in the equine for several years in the treatment of tendon, ligament and joint disease.

IN the USA thousands of dogs have been treated for arthritis using autologous ADMSC's [5].

Allogeneic MSC

Allogeniec cells are processed in a similar manner to autologous. The SVF is cultured and the stem cells isolated and expanded so that millions of cells may be harvested for use. The cultured cells can be frozen in liquid nitrogen and then revived and refreshed ready for use. Once prepared for use the cells survive for 3-4 days at 5 degrees centigrade.

Application of AD-MSCs to small animal practice

1. Osteoarthritis
2. Atopic dermatitis
3. Cardiac disease
4. Renal disease
5. Cognitive dysfunction

Arthritis

One of the most common conditions treated in canine and feline practice.

Monash Veterinary Clinic Trials

In 2009 trials using ADMSCs were commenced.

ADMSCs were sourced from donor or euthanasia dogs. The common source of adipose tissue is around the falciform ligament.

The cells were extracted and cultured at the Monash University Immunology and Stem Cell laboratory (MISCL) under the direction of Professor Richard Boyd [4].

A safety trial was carried out using 5 dogs. ADMSCs were injected IV and the dogs monitored for health, haematological and biochemical abnormalities over a 6 week period. No abnormalities were noted.

5 dogs were selected with OA of the stifle. All dogs received one IA injection of 10 -15 million AD-MSCs. Monitoring was by veterinary and owner assessment. Black et al [1] noted that owner assessment was closely correlated to veterinary assessment. All 5 dogs responded well to the treatment and are still not lame today.

Commercial use of ADMSCs

Public pressure stimulated the trials to move to commercial use in 2011.

Initial cases seen were of a wide variety of conditions. Several cases were treated at the owners request rather than from knowledge of potential efficacy. This did provide the ability to more accurately define the prognosis for most cases and to define the most efficient treatment regimes for individual cases.

Cases seen

- Degenerative myelopathy
- Lumbar and lumbar sacral cord compression
- Cervical pain
- Unilateral and poly arthritis, the most common joints being hips, stifles and elbows.
- Intervertebral disk disease
- Auto immune poly arthritis
- Neoplasia

Case assessment

Most cases received a work up which included a clinical examination, blood and biochemical profiles and radiographs. Many cases seen were not treated with MSCs.

Several arthritic dogs required only improved pain management using NSAIDS, nutraceutical medications, weight loss and pentosan polysulphate. Many owners were happy with that level of treatment. Some cases were commenced on nutraceutical, pentosan polysulphate medication and aggressive weight loss before MSC therapy. Several dogs had neoplasia and were not considered MSC candidates. Several of the treated dogs were very lame, had severe radiographic OA and reduced ROM of affected joints.

The response to treatment is assessed subjectively by the veterinarian and the owner. Essentially the assessment is based on.

- Is the patient better or worse?
- Is the patient less lame?
- Is the patient brighter and more active?

A quality of life scoring system was used to quantify changes.

1. Significantly improved from before treatment
2. Mildly improved
3. No change
4. Mildly decreased
5. Significantly decreased

In some cases lameness scores, ROM assessments, pain on movement assessments, changes in muscle tone and mass were recorded.

Difficulty with case assessments.

Diagnosing lameness due to 1-2 arthritic joints is reasonably straight forward. Dogs that are affected with OA in multiple joints and have concurrent myofascial pain, spinal pain, nerve deficits or reduced ROM of the spine present a greater diagnostic challenge and difficulties when planning ADMSC therapy.

In general practice dogs which present with multiple problems are treated conventionally using nutraceuticals, pentosan polysulphate, NSAIDS and corticosteroids. Whilst most of these cases will respond to this “blanket approach”, the use of ADMSC’s demands a greater level of diagnostic accuracy and therapeutic care. Many dogs which have received ADMSC’s IA have been required to start or continue with more conventional therapy in order to maintain the dog in a state of low to reduced pain management.

Treatments

Treatment modalities are intravenous, intra-articular or a combination of both. The majority of dogs have been treated with IA MSCs. Upto 6 joints have been treated at a time. Currently some dogs affected with hind leg ataxia are being treated with intrathecal injections.

IA injections are given under a GA using Alphaxalone or Propofol. Thiopentone is not used because it is potentially toxic to the MSCs. IV injections are given via an IV catheter placed in the cephalic vein. The cells are diluted in 20 -100 mls of saline or phosphate buffered saline and administered slowly over 10 -20 minutes.

All patients continue to receive any current medication. NSAIDS and corticosteroids are stopped for 5-7 days before and after the administration of MSCs. After treatment the dog’s exercise is restricted to lead work for 7 days then slowly increased avoiding vigorous

exercise. The need for ongoing NSAID therapy, nutraceuticals and pentosan polysulphate is regularly assessed and in some cases can be reduced or stopped.

Dose Rates

1. Intra-articular: Dose is limited by the available joint space. A dose of 0.5 ml containing 10 million cells is used for small dog joints (<20kg), and 1.0 ml containing 15 million cells for large dog joints is used.
2. Intravenous: Dose used has been calculated on both weight and surface area.

Less than 10 kg	20 million cells
10 -20 kg	40 million cells
20 - 30 kg	60 million cells
30 – 40 kg	80 million cells
40 – 50 kg	100 million cells
Greater than 50 kg	120 million cells

Results

1. **Intravenous therapy**
   Most dogs treated were old, polyarthritic, affected with lumbar and lumbar sacral spondylosis with varying degrees of hind leg conscious proprioceptive deficits and varying degrees of CCD.
   These cases have not responded as well as intra articular cases. However almost all clients report that following IV MSC therapy their pets are happier and brighter! This response occurs quickly within the first 7 days.
   It is not known if this is due to reduced pain levels or improved cognitive function.

Total Number	20	Percentage
Significantly improved	3 *	15%
Mildly improved	11	55%
No response	5	25%
Died before assessment	1	5%

   Note * includes one case if IVDD

2. **Intra-articular injection**
   Over 30 cases have been treated and the response is excellent. Most dogs respond within 1-2 weeks. Some dogs take longer to respond and any improvement may continue for upto 12 weeks. If there is no response by 12 weeks then none can be expected.

Total Number	32	Percentage
Significantly improved	16	50%
Mildly improved	13	46%
No response	2	6.3%
Died before assessment	1	3%

   Stifles OA
   Stifle OA cases consisted of
   1. Stifles which had had surgery for anterior cruciate ligament (ACL) injury
   2. Cases of stifle inflammation with or without joint laxity due to ACL injury.

   The stifle joint responds well to IA MSC therapy.

Cases of stifle inflammation and ACL instability require special care. Most cases do respond well, but some cases will proceed to a complete ACL rupture requiring surgery. There is no way to predict if this will happen.

15 cases involving stifle OA were treated and 5 were bilateral. None of the cases which proceeded to rupture were bilateral conditions.

Total Number	15	Percentage
Significantly improved	10	66.6%
Mildly improved	5	33.3%
No response	0	9%

Elbow OA

11 cases of elbow OA were treated of which 5 were bilateral. Many of these elbows had had previous surgery. Some elbow cases have not responded well.

Total Number	11	Percentage
Significantly improved	4	36%
Mildly improved	5	45%
No response	1	9%

Hip OA
Hip OA responds well to MSC therapy

Total Number	7	Percentage
Significantly improved	6	86%
Mildly improved	1	14%

Adverse reactions.
Some dogs may be a little stiff for 48 hours after IA injections. They have a normal recovery.
One case of joint swellings has been reported post IA injections. The Dog did not fully recover.
One case of ataxia after rapid IV injection has been recorded. The ataxia lasted 30 seconds and the dog recovered uneventfully.

Conclusion
ADMSC therapy is an exciting method of treating OA and inflammatory conditions of the musculoskeletal system. It offers a therapy which can reduce or eliminate the need for conventional arthritis treatments. It offers a therapy which is more supportive and healing for the joint tissues compared to conventional treatments.

References

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