Small Animal Regenerative Therapy

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Regenerative therapy in small animal surgery has become a commonly accepted treatment modality. Indeed, augmenting the treatment of many conditions via regenerative practices has demonstrated both efficacy and safety for patients with numerous clinical conditions. Understanding how stem cells and platelet-rich plasma work in injured tissue is necessary to determine the appropriate use of these processes. Greater understanding of the mechanisms of action of these treatments through research will only further enhance our ability to use regenerative therapy to treat a greater variety of ailments.

MSCs

Stem cells are typically grouped into three categories:

- Totipotent stem cells. These are embryonic stem cells that exist in the first few cell divisions of the early developing embryo following fertilization. These cells, along with extra-embryonic cells (*e.g.* placental cells), develop into the various body tissues in the developing embryo.
- Pluripotent stem cells. These are developing embryonic cells that give rise to other cell types that make up tissue.
- Multipotent stem cells. These are adult stem cells. They possess the ability to develop into more than one cell type but are more limited than pluripotent cells, which have a greater potential for differentiation into numerous tissue types. It is multipotent stem cells that are most often used clinically for a variety of types of biological applications.[1]

Mesenchymal stem cells (MSCs) are multipotent stem cells circulating throughout the bloodstream and tissue, serving multiple functions. These cells exist in high concentration in a variety of tissue types, including, among others, bone marrow and adipose tissue.

MSCs function in multiple ways:

- Secrete trophic factors (cytokines and chemokines)
- Have anti-inflammatory effects
- Differentiate into tissue
- Home in to the site of injuries
- Modulate the immune system

Growth factors and cytokines are secreted by stem cells in response to disease or injury. Cytokines and trophic factors, such as Interleukin, Prostaglandins, transforming growth factor-β (TGF-β), and others, modulate the local immune response and regulate inflammatory reactions. These trophic factors are important in recruiting other cells, which aid in diminishing tissue injury, promoting neovascularization, recruiting other stem cells for tissue repair, and inhibiting fibrosis.

Chronic inflammation
One of the functions of MSCs is to reduce inflammation in tissue when present. Due to this mechanism, stem cell therapy is an attractive method of treatment for chronic inflammatory conditions, such as osteoarthritis (OA) in dogs. Basically, mesenchymal stem cells accomplish this by increasing the concentration of anti-inflammatory mediators at the site of inflammation, while decreasing pro-inflammatory mediators concurrently. Anti-inflammatory mediators, such as Interleukin-10 and Interleukin-4, increase in response to inflammation by MSCs. Pro-inflammatory mediators, such as Interferon-γ and Tumor Necrosis Factor-α (TNF-α), are decreased by stem cells in response to inflammation. The cumulative result of this is an anti-inflammatory effect in the face of tissue inflammation.

This is one of the primary mechanisms of interest in the use of MSCs in the treatment of patients with OA. By reducing inflammation and promoting tissue repair, the magnitude of inflammation is generally reduced over time and the regenerative phase of tissue repair is upregulated—thus also reducing both fibrosis and scar tissue formation.

Treatment of dogs with chronic OA using stem cell therapy has become an attractive treatment option. Case selection for patients with osteoarthritis, however, is important. Patients with orthopedic injuries able to be repaired should first undergo this surgery. Further, conditions such as a cranial cruciate ligament (CCL) injury or a fragmented coronoid process (FCP) should have these disorders addressed by surgery as the primary treatment.

Patients that are lame with secondary osteoarthritis due to injuries (*e.g.* previously repaired CCL injury, hip dysplasia, etc.) are more suitable stem cell treatment candidates. Clients must be made to understand stem cells are not expected to re-grow damaged or torn tissue; rather, the target of this modality is the process of decreasing chronic inflammation.

Other factors to consider when determining ideal cases for OA treatment with stem cells include:

- the presence of mild-moderate osteophytosis;
- lack of other known systemic diseases (especially neoplasia);
- presence of a normal neurologic exam; and
- lack of response to appropriate medical management for their arthritis.

Owners must have rational expectations of what should be expected in cases when treatment is initiated. The best-case scenario for treatment expectations involves improvement in patient quality of life (QoL), reduced pain and discomfort when moving, and decreased necessity for other medications (typically non-steroidal anti-inflammatory drugs [NSAIDs]).

Arthritic patients treated successfully with MSC therapy can hope for a six- to eight-month period of improvement in their clinical signs. Given this is an intermediate-lasting therapy, re-injection of joints with MSCs is expected as-needed. Shah et al. demonstrated that 90 percent of arthritic dogs younger than nine years of age that were treated with MSC therapy showed excellent improvement in pain and mobility, while 60 percent of dogs 10 years of age and older showed good improvement.[2]

Canine OA treatment process

Stem cells circulate through the bloodstream and are present in many tissue types; however, the highest concentration of mesenchymal stem cells in dogs are present in normal adipose tissue. As such, locations of adipose deposition (*e.g.* falciform fat, lateral thoracic body wall fat) are typical sites of harvest for the purpose of accumulating MSCs to be used for treatment.

A minor surgical procedure to collect this adipose is, therefore, necessary to acquire these samples. For processing through Vet-Stem, Inc., in Poway, Calif., for example, 60 grams of adipose tissue is typically harvested. The adipose is stored in phosphate-buffered saline tubes and shipped overnight to the lab for processing. After extraction of MSCs from the adipose tissue, some cells are shipped back for treatment, while others are banked in frozen storage.

After cells are received, inflamed joints are injected intra-articularly while the patient is under heavy sedation (this author prefers to use a combination of dexmedetomidine and butorphanol). There are also bench-top in-hospital units that perform the function of washing out the stem cells for injection following the extraction of tissue. Stem cell service companies are usually able to bank multiple samples for future injections, along with adipose tissue for future cell culture. Samples may even be collected pre-emptively in patients undergoing elective surgery for banking and future use in cases when a pet is deemed likely to develop arthritis due to injury, breed, or conformation.

In general, this author has seen great improvement in the comfort and mobility of arthritic patients, usually within 7 to 10 days following the first joint injection. The criteria for assessment of improvement are usually subjective, relying on owner observation and input.

Clients typically note dogs who respond positively show:

- improved ability to get up after rest;
- increased comfort with movement;
- ability for longer duration of exercise; and
- less requirement for rescue medications, such as NSAIDs.

With subsequent injections, there is an observed more rapid response and improvement in comfort and mobility, usually within three to five days after injection. The duration of positive effect from articular stem cell injections for the treatment of OA is variable but may last approximately six to eight months before re-treatment is necessary. One advantage of treatment with stem cell therapy is that multiple joints may be treated at the same time. Cells may also be injected intravenously (by prior arrangement before processing of cells) if there are other systemic needs for stem cells.

PRP

Platelet-rich plasma (PRP) is an ultrafiltrate of plasma containing a very high concentration of platelets.[3] Though we most commonly think about platelets solely for their coagulative abilities, these cells also contain growth factors and cytokines, such as transforming growth factor-β (TGF-β), platelet-derived growth factors (PDGF), and other factors important in stimulating tissue healing. TGF-β accelerates production of collagen and is chemotactic for pre-osteoblasts, which can stimulate bone healing, while PDGF activates macrophages and stimulates mitogenesis and angiogenesis. Platelet half-life is five to seven days, and the growth factors released by platelets are continually released over this time.

PRP can be used for musculoskeletal trauma and cutaneous injuries. This plasma is often used as an adjunct to treatment of tendon injuries (with or without surgical repair or injected directly into the tendon via ultrasound guidance). Platelet-rich plasma also contributes to the anti-inflammatory effects of stem cells when injected intra-articularly for the treatment of OA.

One of the most common uses for PRP is as an adjunct to the treatment of calcaneal tendon ruptures. Following surgical repair, PRP is injected at the time of surgery around the tendon. Ultrasound-guided injections of biceps brachii and supraspinatus tendon injuries are other common injuries that PRP has shown beneficial effects. Platelet-rich plasma is also used to augment bone grafts when delayed bone union of fractures is suspected.

Treatment of canines with a combination of stem cells and PRP was demonstrated to improve weight bearing in 55 dogs with supraspinatus tendinopathy in one study.[4] Other studies have demonstrated similar efficacy of PRP +/- MSC for soft-tissue injuries in smaller case studies. There is great room for further research in this area as we learn more about stem cell and PRP mechanisms of action.

Additional potential applications

Future direction for regenerative therapy research includes treatment of neurologic injuries, acute and chronic renal disease, hepatic injury, ophthalmic applications, dental applications, accelerated bone healing, and more. Indeed, there is a tremendous amount of ongoing research for human and veterinary applications. While there is a great deal we do not yet know or understand with respect to regenerative therapy, this is certainly an exciting area for future therapeutic applications.

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