Equine Regenerative Rehabilitation

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Abstract

Regenerative or Biologic Medicine encompasses both cellular and non-cellular therapies. Regenerative Rehabilitation encompasses the use of Regenerative or Biologic Medicine in conjunction with Rehabilitation. This presentation covers the basic types that are utilized today and in what circumstances they may be utilized. It will discuss when to use these therapies in relation to the rehabilitation process. It will also discuss current FDA regulations on the use of cell based products in Veterinary Medicine.

Keywords

Regenerative Medicine, Biologic Medicine, Rehabilitation, FDA Regulations

Cell Based Therapies

Embryonic stem cells - The embryonic stem cell is present at approximately one week after conception. These cells are pluripotent, meaning they can become any type of cell that is present in the adult. These are not widely available for equine use.

Adult-derived stem cells - Stem cells can be found in nearly all tissues throughout life. They are used for normal remodeling and repair. However, the older these cells become, they don't multiply (expand) as well in culture and they don't differentiate into different types of cells as easily. Mesenchymal stem cells (MSC) are basically adult-derived stem cells derived from just

about any tissue in the body. They usually from bone marrow or fat tissue. Once isolated from the donor tissues and expanded, the stem cells can be used to treat a variety of different tissues. Although the cells isolated from fat and bone marrow are MSCs, there may be differences. Bone marrow derived have been the most thoroughly studied and have the most evidence for the ability to undergo chondrogenesis, tenogenesis, and osteogenesis. They may also have efficacy in treating soft tissue injuries within joints.

Autologous stem cells - Autologous means that the cell or tissue used in a patient is from that individual, or is self-derived. There's minimal risk for disease transmission. The isolation and expansion process takes approximately three weeks. Besides the length of time it takes to culture and expand the cells, the other negative with autologous cells is that all stem cells are not created equally. For instance bone marrow derived cells from one individual may be inferior in quality compared to the exact same cell obtained from another individual.

Allogeneic stem cells - Allogeneic means the cell or tissue used in a patient is from a different individual of the same species. Allogeneic MSCs would be considered an "off-the-shelf" option. The advantage of these cells would be that they are available within a short period of time and they have been fully characterized so that their quality is known. Allogeneic MSCs are considered essentially the same as a drug by the FDA, and as such the FDA would require the same safety and efficacy studies required for pharmaceuticals (see attached guidelines from AAEP).

Adipose Derived Cellular Material - A rich source of pre-adipocytes, MSC's, endothelial progenitor cells, T cells, B cells, mast cells as well as adipose tissue. The freshly isolated heterogeneous cell fraction, isolated from native adipose tissue or liposuction aspirates is called the Stromal Vascular Fraction (SVF). It has been available for years. Fat is collected, processed

and the cellular homogenate is available for use in approximately 24 hours (48 hours if processed off site). As with any adult tissue, stem cells may be isolated and expanded from fat. These are termed adipose-derived stromal cells (ASCs) and are a homogeneous, plastic adherent cell population.

Autologous Fat Injections – Subcutaneous fat is collected and micro-fragmented. This fat suspension is then injected into the diseased or injured structure. Provides adipocytes, reticular cells, progenitor cells, and perivascular cells (cells surrounding micro-capillaries).

Bone Marrow Concentrate – This is made by concentrating a bone marrow aspirate (BMA). It is a biologic concentrate derived from a patient's own bone marrow. Concentrated BMA is high in mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), which are known to be critical in biological processes such as tissue regeneration and bone formation. The numbers of MSC's are low compared to MSC's that are obtained by culturing the bone marrow. It does contain other hematopoietic cells and growth factors. Available as a stall side product that can be utilized in a short period of time.

Non-cellular regenerative medicine

Platelet-Rich Plasma (PRP**) -** Platelets can release many bioactive substances that promote healing, stimulate blood vessel formation, recruit endogenous stem cells and control inflammation. PRP is plasma with a platelet count above that of whole blood. It can be done patient-side for immediate use, which means it's a relatively inexpensive approach.

There are three general forms. Each of which has their proponents and opponents. These forms include pure PRP, leukocyte rich PRP, and leukocyte-reduced PRP. This reflects the ratio of platelets to leukocytes in the final product. Currently the "ideal" makeup is undefined. It is thought though that a higher concentration of platelets would mean more growth factors are

available for healing. There has been concerns expressed that increases in leukocytes may cause an increased inflammatory response following injection. However, it is also known that leukocytes produce Interleukin 1 Receptor Antagonist Protein (IRAP) so they may also have some benefit. An Advantage PRP's is that it is autogenous, rapidly prepared and it can be frozen. However, freezing may damage leukocytes and activate some of the platelets. Fresh PRP can be used to form a clot in an injury if thrombin and calcium are added.

Autologous conditioned serum (ACS) - ACS is used to upregulate interleukin-1 receptor antagonist protein (IL-1RA; IRAP) to block the activity of interleukin-1. Inhibiting interleukin-1, reduces inflammation at the injured or inflamed site. Commercially available kits are available that utilize whole blood. These involve incubating blood with medical-grade glass beads that cause leukocytes (white blood cells, specifically the macrophages) and platelets to produce and release endogenous substances, such as IL-1RA. There are also other growth factors that are in ACS that may also contribute to its action. The process usually takes about 24 hours, though there are some kits available that only take 1-2 hours.

Growth Factors Therapy - Involves the introduction of these factors directly by injecting proteins, or indirectly by using gene therapy techniques to stimulate protein production. TGF-β (transforming growth factor-β) and IGF (insulin-like growth factor) have been injected in horses as proteins or used as gene therapy to stimulate healing of hyaline cartilage (normal cartilage within the joint) and tendon, respectively. Bone morphogenic protein (BMP) has been injected into horses as proteins or used as gene therapy in fractures and cyst-like lesions to stimulate exactly what you might expect: bone production. Growth-hormone-releasing hormone (GHRH) gene therapy has been used to treat laminitis. IL-1Ra gene therapy has been used to treat joint inflammation.

Regenerative Medicine Uses

At this time the primary use for Regenerative Medicine in the horse is for musculoskeletal disease. As a very general rule cellular therapies are primarily used for soft tissue injuries (tendon, ligaments and meniscal). There is ongoing research investigating cell based therapies combined with different matrixes for cartilage, bone and wound healing.

Platelet rich plasma is utilized for both soft tissue and joint injuries. PRP is useful for acute tendon and ligament injury and is often injected intralesionally with ultrasound guidance. However it has been found to be useful in chronic injuries that do not have major defects.

ACS is primarily utilized for the treatment of acute joint inflammation. It is most useful in inflamed joints that do not demonstrate significant signs of osteoarthritis.

Cell-based Products for Animal Use – Guidance for Veterinarians provided by AAEP

As you may or may not be aware, the FDA issued their final guidance on cell-based products for animal use late last year. (Guidance for Industry Cell-Based Products for Animal Use #218) http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm450556.htm

The AAEP convened a task force to review the information and provide a synopsis for the membership to better understand these regulations as they pertain to the use of these products. The following points represent these views.

1. FDA will regulate stem cell products for use in animals. Historically the USDA has regulated many common biological products used in animals. This will not be the case when it comes to cell based or stem cell products—the FDA will be the federal regulatory agency in most cases.
2. As it relates to product approval and surveillance, the FDA will regulate animal cell-based products (ACPs) as new animal drugs.

3. In general, as a new animal drug, the ACPs must be evaluated for safety and efficacy for use as described by the label. The manufacture of these products must meet the requirements of current good manufacturing practices (cGMP). These products will be required to go through the New Animal Drug Application (NADA) process.
4. Type I ACPs include autologous (self to self) products which have been processed or "more than minimally manipulated" - this includes cell expansion and cell differentiation. The finished ACPs may be combined with or modified by adding another drug or device. Cells used for allogeneic (donor and recipient are not the same individual, but the same species) or xenogeneic (different species) use also fall under Type I ACPs whether they are manipulated or not. Non-homologous applications will also be Type I. For example, adipose tissue used in treatment of tendon disease would be non-homologous use.
5. Type II ACPs are autologous (self to self) products which are only minimally manipulated, and are for homologous use (cell function is the same in the donor tissue as in the recipient tissue), and in which the manufacturing process does not involve combination with anything except water, crystalloids or a sterilizing, preserving or storage agent, and the finished ACP is not combined with or modified by the addition of a drug or device. If the manufacturer meets these criteria, including Good Manufacturing Practices, then the agency will place a low priority of oversight for Type II products. It is the expectation that only a small number of products will meet these criteria as Type II ACPs.
6. Type I ACPs will come under more stringent regulatory scrutiny and have a higher enforcement priority as compared to Type II ACPs.
7. All facilities which manufacture ACPs (either Type I or Type II) must register with the FDA and drug list their products and all facilities must be GMP compliant. This includes

patient-side kits that are used for Type I and Type II ACPs. Sponsors of these products should report adverse events. All labeling and promotion must be truthful and include relevant hazards and precautions. Sponsor must submit drug experience reports, all safety and efficacy information and all data from all studies.

8. Both Type I and Type II ACPs cannot be marketed (sold) until they are an approved New Animal Drug (NAD). Type II products will be viewed with a lower oversight/enforcement priority as compared to Type I products.
9. Both Type I and Type II ACPs (which are in the approval process) can be used in investigational new animal drug (INAD) studies, but not sold.

The above is intended to guide with regard to use of stem cells in equine veterinary practice. These points reflect the sentiments of the FDA and veterinarians are encouraged to comply with these guidelines when using these products.

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