Stem Cell Therapy

Stacey Oke, DVM, MSC

Not All Equine Stem Cells Equal: Choose your “Weapon” Wisely

Practitioners have identified stem cell therapies as potentially powerful weapons in the war on wounds and injuries. But the exact cell types most beneficial for certain “battles” remain unclear. “Stem cells, particularly a specific cohort of stem cells called mesenchymal stromal cells that have the ability to become any one of a variety of cell types such as bone or cartilage, are a promising tool for treating various orthopedic conditions,” explained Janina Burk, DVM, of the Large Animal Clinic for Surgery at the University of Leipzig, in Germany. She and her colleagues recently compared five different mesenchymal stromal cell sources.

Mesenchymal stromal cells can be derived from bone marrow, fat (adipose) tissue, other body tissues (such as tendons, the body tissue used in this study), and umbilical cord blood and tissue. Currently, the “best” source of stem cells for treating tendon, ligament, and other orthopedic injuries remains a topic of great debate due to a lack of comparative studies. And while many researchers are running from bench to stall with stem cell technology, others are recalling their troops and holding out for the “hero” stem cell source.

In their study the team determined:

- The highest cell yield was from adipose and tendon tissues;
- Those two populations of cells grew quickly in culture as compared to the other cell populations;
- Normal tendon tissues’ production of cell “markers” varied depending on the cell source (meaning not all mesenchymal stromal cells behaved like true tendon cells); and
- Cells’ ability to differentiate into bone or cartilage cells differed. Bone marrow-derived cells were best at becoming bone cells, and cells from umbilical cord blood were best at becoming cartilage cells.

“The five different populations of stromal cells used ... showed significantly distinct properties in this study, suggesting that cell source could play a major role in the behavior of stem cells used in the clinical setting,” Burk concluded. “It is possible that exploiting these properties could improve the outcome of stem cell therapy.”

In other words, either choosing a specific cell source or altering the way mesenchymal stromal cells are treated in the laboratory before injecting them into an injured tendon could maximize this therapy’s efficacy, improving injured horses’ chances of recovering and returning to work.

Mesenchymal stromal cells have the ability to become a variety of cell types, such as bone or cartilage.
Tweaking Stem Cell Therapy Use

“To derive the greatest benefit from stem cell therapy, we need to optimize and describe (stem cells’) behavior in the laboratory,” said Mandi J. Lopez, DVM, MS, PhD, Dip. ACVS, director of the Laboratory for Equine and Comparative Orthopedic Research, in Louisiana State University’s Department of Veterinary Clinical Sciences. “Ultimately, this is necessary for the preclinical studies to confirm safety and efficacy prior to controlled clinical trials.”

In their study, Lopez and colleagues isolated “multipotent stromal cells” from bone marrow and adipose tissue. Multipotent stromal cells are immature cells found in adult tissues that are thought to maintain normal tissue and respond to injury by maturing into adult cells as needed. In this sense they are a form of stem cells; however, unlike “totipotent” stem cells (that can become any tissue type), multipotent stem cells are generally limited to becoming the type of tissues from which they’re derived. For example, a mesenchymal stromal cell can become muscle or bone cell but not a brain cell. In the study by Lopez et al., veterinarians harvested cells from adipose tissue and bone, so these cells were able to become tissues such as bone, adipose tissue, and cartilage.

“The ability to isolate, grow, and selectively increase the number of these cells in the laboratory, as well as their ability to become different cell types, has been confirmed many times,” Lopez said. “The focus (of research) has now begun to shift toward determining the best ways to use the cells to meet the needs of clinical patients.”

One way to do this is to use patient cells to grow new or “neo” tissues in the laboratory. In theory, the new tissue can then be applied to either treat or replace damaged tissue, similar to a graft.

The team looked at the ability of multipotent stromal cells from bone and adipose tissue to become bone, adipose, and cartilage cells, and their ability to produce “neotissue” after the scientists loaded these cells onto pieces of collagen, called a scaffold (they did this using a perfusion bioreactor, which Lopez said “moves the cells suspended in fluid through the scaffold ... to equally distribute the cells and maximize the number of cells on the collagen”). The scaffold provides the framework to which the cells adhere and begin producing tissue.

After loading, the researchers maintained the cells on the scaffolds in growth conditions for seven, 14, and 21 days. Then they evaluated the number of live cells, distribution in the scaffold, gene expression, and neotissue formation.

“Cell source could play a major role in the behavior of stem cells used in the clinical setting.”

— DR. JANINA BURK

“...to have earlier expression of bone and cartilage genes, while adipose tissue cells had earlier expression of adipose genes.

“This may mean that multipotent stromal cells from both types of tissue may be used for tissue regeneration under similar laboratory conditions,” noted Lopez.

Additionally, the researchers determined that the perfusion bioreactor provided an efficient and effective way to load the cells onto the scaffold, potentially minimizing the total number of cells scientists need to generate specific neotissue.

She concluded, “These findings support our ongoing efforts to develop equine stem cell tissue regeneration to provide new and improve upon existing treatment options, especially in the area of fracture repair.”
**ANTIPROTOZAL PELLETS**  
**FOR OVAL, CILIUM, AND RICH SPORE EPITHELIAL PROTOZOA (SPP) CAUSED BY Sarcocystis neurona**

**DESCRIPTION**  
Cultured drug for the treatment of equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona. PROTAZIL® (diclazuril) is supplied as oral pellets containing 1.56% diclazuril, a molecular formula of C_{28}H_{37}N_2O_3, molecular weight of 407.5 g/mol and a molecular structure as follows:

![Molecular Structure of Diclazuril](image)

**INDICATIONS**  
PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets are indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona in horses.

**DOSAGE AND ADMINISTRATION**  

**DOSAGE**  

**Adult Horses**

- For the treatment of EPM caused by *S. neurona* in adult horses, PROTAZIL® (1.56% diclazuril) is administered as a dose of 1 mg/kg (0.45 mg/lb) of body weight for 28 days. The horses were then evaluated for clinical changes via a modified Mayhew neurologic scale on Day 48 as follows:
  - 0: Normal gait
  - 1: Normal gait; occasional loss of step
  - 2: Normal gait; increased stride
  - 3: Truncal swaying
  - 4: Neurological deficit is profound at normal gait: horse frequently stumbles or trips and may stumble or walk on all four legs
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- Each horse's response to treatment was compared to its pre-treatment values. Successful responses were defined as a change of at least one grade in the modified Mayhew neurologic scale on Day 48 ± 2 days. The referring veterinarian reported that the horse had been fed grass clippings containing diclazuril (0.1 ng/mL diclazuril and greater than 95% inhibition of merozoite production (IC 95)) was treated by the veterinary and the infection was resolved.

- For the treatment of equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona in horses, PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets with concomitant therapies in horses has been clinically effective in reducing the severity and duration of clinical signs and symptoms for 28 days. The horses were then evaluated for clinical changes via a modified Mayhew neurologic scale on Day 48 as follows:
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  - 1: Normal gait; occasional loss of step
  - 2: Normal gait; increased stride
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**ADVERSE REACTIONS**

- There were no test article-related findings seen during the study.

**CONTRAINDICATIONS**

- Use in horses with a history of disease or predisposition to disease that may be exacerbated by treatment with PROTAZIL® (diclazuril) Antiprotozoal Pellets.

**PHARMACOKINETICS IN THE HORSE**

- Diclazuril is an unscheduled (protoparacarbamate) compound with a toxicity similar to the tricyclic antidepressants. PROTAZIL® (diclazuril) is supplied as oral pellets containing a molecule that can be mixed into a top-dress in feed. In treated feed, diclazuril is ingested by the horse along with its normal feed intake. Diclazuril is not metabolized by the horse but is excreted unchanged in the feces. Diclazuril is not detectable in the feces of treated horses.

**REFERENCES**


Recognizing EPM can be hard. Now the treatment is easy.

Introducing Protazil® (1.56% diclazuril), the safe, easy way to treat EPM. Protazil is the first FDA-approved alfalfa-based pelleted EPM treatment that makes accurate dosing and administration simple.

Ask your veterinarian for Protazil. Because we’re for helping you help your horse.
Visit us at Protazil.com

Protazil is contraindicated in horses with known hypersensitivity to diclazuril. Safe use in horses used for breeding purposes, during pregnancy, or in lactating mares has not been evaluated. The safety of Protazil with concomitant therapies in horses has not been evaluated. See related page in this issue for details.