Employing Recombinant Adeno-associated Viral Vectors for Delivery of a Therapeutic TIMP-3 Transgene to the Equine Distal Extremity Using a Clinical Regional Limb Perfusion Technique.

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Background

Laminitis is the most common cause of lameness and the second most common reason for euthanasia in horses. Though the onset of laminitis is multifactorial, this crippling disease is characterized by the breakdown of the lamina, the separation of the coffin bone from the hoof wall, and the eventual downward rotation of the coffin bone causing extreme pain. The epidermal and dermal layers of the hoof are stabilized by connective tissues partly composed of proteoglycans known as aggrecans. In the healthy lamina, a group of proteins known as aggrecanases breakdown aggrecan. Tissue Inhibitor of Metalloproteinases (TIMP-3) inhibit excessive aggrecanase activity. During laminitis, aggrecanase production is upregulated.

Currently, there are no effective prevention or treatment strategies for laminitis. The limited access nature of the deep hoof tissues makes laminitis a good candidate for gene therapy. This gene therapy study uses a viral vector (rAAV) to transduce lamellar target cells with a therapeutic gene (TIMP-3 gene). The expression of the TIMP-3 gene will produce the TIMP-3 protein. Gene therapy using rAAV TIMP-3 should allow the lamellar tissue to resume homeostasis between aggrecanases and TIMP-3.

Previous studies have determined optimal vectors, promoters, serotypes, and surfactants for successful transduction and distribution of marker genes. The in vivo studies administered the vector using lateral Regional Limb Perfusion (RLP) which requires general anesthesia. The current study uses a standard veterinary technique, standing RLP, in which the horse is only mildly sedated. This study will apply principles learned from parent studies to deliver the therapeutic gene TIMP-3 deep into the equine hoof.

Objectives and Hypothesis

Objectives
- Using marker genes, compare standing RLP and lateral RLP distribution patterns in vivo.
- Using TIMP-3 genes, determine TIMP-3 gene and protein biodistribution and intensity in vivo.

Hypothesis
- Standing RLP will have a similar marker distribution to lateral RLP.
- rAAV TIMP-3 biodistribution pattern will be similar to rAAV marker distribution.

Methods

Regional Limb Perfusion

Viral Injection
- The horses receive an anti-inflammatory, Phenylbutazone.
- Hoof samples are collected after 28 days.
- Blood samples are collected before injection and before hoof collection for antibody comparison.
- One horse was injected with a marker gene.
- One horse was injected with a vector mixture of marker genes and TIMP-3 genes.

Lab Tests
- The hoof samples will be analyzed using β-Gal staining, quantitative real-time polymerase chain reaction (qPCR), western blot, and immunochemistry (IHC) tests.

- Evaluates the distribution of protein expression
- Stains marker proteins red

- Gel electrophoresis
- Evaluates the distribution of transduction
- Detects and quantifies the vector

- Gel electrophoresis chemiluminescence, radiograph film exposure.
- Detects concentration and occurrence of TIMP-3 proteins in the lamellar tissue samples

Results

Figure 1: The degradation of the lamina causes the rotation of the distal phalanx.

Figure 1: Palpating lateral digital vein for standing regional limb perfusion.

B-Gal Staining

qPCR

Western Blot

IHC

References