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.

**Background**

Laminitis is the most common cause of lameness and the second most common reason for euthanization 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 proteinases known as aggrecanases breakdown aggrecan. Tissue Inhibitor of Metalloproteinases (TIMP-3) inhibit excessive aggrecanase activity. During laminitis, aggrecanase production is upregulated.

**Objectives and Hypothesis**

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- 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.

**Results**

**A**

Lateral Regional Limb Perfusion Dose Effects on Distribution

**B**

Preliminary Standing Regional Limb Perfusion Transduction

**Conclusions**

Because of similar distribution patterns of transduction and expression of marker rAAV vectors, Standing RLP is an appropriate replacement technique for lateral RLP. Using standing RLP to deliver the therapeutic vector will allow veterinarians to administer this treatment using a standard clinical procedure.

If the hoof successfully transduces and expresses the TIMP-3 gene, there will be sufficient evidence to allow for future studies evaluating the effects of gene therapy on clinical cases of equine laminitis. This could lead to the first effective preventative therapy against laminitis.

**References**
