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 proteinases 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 Profusion (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

Standing Sedation → Local Anesthetic → Tourniquet

Remove Tourniquet and Catheter → Inject Lateral Digital Vein → Catheter

Viral Injection

Inject 15 mL of rAAV

Unload carpus

Inject 15 mL of rAAV

Flush Catheter

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.

B-Gal Staining

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

qPCR

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

Western Blot

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

IHC

• Immunostaining
• Examines for intensity and distribution of the TIMP-3 protein
• Protein localization

Results

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

Figure 2: rAAV copy number assay. We performed qPCR of the transduced tissue regions (A–I) to detect the number of viral genomes incorporated into each region of hoof tissue. A) The original injections were performed with the horse in lateral recumbency under general anesthesia. B) The current experimental injections were performed using a common clinical standing regional limb perfusion with the horse sedated.

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
