



Platelet-Rich Plasma:

A New Treatment for Tendon and Ligament Injuries in Horses

Andris J. Kaneps, DVM, PhD, Diplomate ACVS

New England Equine Medical and Surgical Center

15 Members Way

Dover, NH 03820

603-749-9111

www.newenglandequine.com

Tendon and ligament injuries are a common cause of lameness in horses. Over 30% of racehorse lameness is due to tendon or ligament injury. Traditional treatment of these injuries often requires 6 to 12 months for rehabilitation. Healing may be incomplete, resulting in a weaker healed tendon. Also, traditional treatments have a 30-50% failure rate.

Because of these factors continued research for new treatment techniques for tendon and ligament injuries is an active area of equine research. In this vein, platelet-rich plasma (PRP) therapy for equine tendon and ligament injuries has been investigated at The Ohio State University College of Veterinary Medicine and is being used in clinical cases at New England Equine Medical and Surgical Center.

What is Platelet-Rich Plasma?

PRP is the result of processing whole blood to obtain the blood fraction that has the highest concentration of platelets. Platelets contain a number of growth factors within their α -granules that are released upon activation.^{1,4} Large amounts of transforming growth factor beta (TGF- β) and platelet derived growth factor and smaller amounts of insulin-like growth (IGF), epidermal growth factor (EGF) and TGF- α are released upon activation from human blood.² These growth factors and others act synergistically to enhance access of healthy inflammatory cells to the area of tissue injury, formation of new blood vessels (angiogenesis), formation of new connective tissue (fibroplasia) and regeneration of skin (re-epithelialization).³ PRP has been used to enhance bone healing, bone-implant security and wound healing.⁵⁻⁸

Methods to concentrate platelets in humans have been adapted to the horse. Dr. Wes Sutter at The Ohio State University evaluated two commercial methods for production of platelet concentrates from equine blood by comparing platelet and growth factor concentrations.⁹ Equine whole blood was collected and processed either by a buffy coat method or apheresis method to produce platelet poor and platelet rich fractions. Packed cell volume, white blood cell count, and platelet count were compared between each method and among the platelet poor, whole blood, and platelet rich fractions. Growth factor concentrations of transforming growth factor beta (TGF- β 1 and TGF- β 2) and insulin-like growth factor (IGF-1) were compared between the two methods, and correlated to platelet counts. IGF-1 has higher concentrations in blood plasma, not in PRP, so was evaluated as a control for the PRP concentration techniques.

Platelet counts were concentrated 9.2-fold by the buffy coat method and 5.2-fold by the apheresis method compared to whole blood values. The apheresis platelet concentrate fraction platelet count was further concentrated by a commercial filtering procedure to 14-fold greater than whole blood. TGF- β 1 concentration in the platelet concentrate was 2.8-fold greater with the buffy coat method and 4.3-fold greater with the apheresis method compared to whole blood. TGF- β 2 concentration was 3.6-fold greater with the apheresis method compared to whole blood. IGF-1 was not concentrated using either method compared to whole blood, verifying that this growth factor is not concentrated in PRP. Platelet concentrations correlated with growth factor concentrations.

This study proved that platelets and TGF- β 1 can be concentrated reliably from equine blood using either method without modification of human protocols. Concentration of platelets from whole blood and platelet concentrate correlated with concentration of TGF- β 1 and, therefore, a comparison of platelet count in the platelet concentrate as compared to whole blood can estimate the concentration of TGF- β 1 in similar fractions. The apheresis method resulted in higher concentrations of TGF- β 2 and IGF-1 than did the buffy coat method, the biologic advantage of which was not determined from our study.⁹

Clinical use of platelets

Injection of PRP is recommended for both recent tendon and ligament injuries and those injuries that have not healed using traditional rest and controlled exercise. We recommend injection of a recent injury 30 days after occurrence. The procedure is done in the standing horse under sedation and local nerve block. Whole blood is obtained from the horse, blood is processed, and the PRP is injected into the tendon or ligament injury. The limb is bandaged for two weeks. The horse is confined for two weeks, then returns to a controlled exercise protocol based on the severity of injury and level of lameness. Re-examination with ultrasonography is conducted every 30 days over the first two months, then every 60 to 90 days during the remaining healing period. In most cases only one PRP injection is needed to result in complete healing of the injury.

We currently process equine whole blood to obtain PRP using a system distributed by Vet-Stem of Poway, California. The GenesisCS system is specifically

made for use in horses and has been validated for concentration of platelets from equine blood. The system is sealed from outside contamination except for the access port that connects to the syringe containing the horse's whole blood.

PRP has been injected in over 200 horses with tendon and ligament injuries. Treatment has resulted in rapid healing of previously static lesions and return to soundness and function. Evaluation of clinical cases is ongoing, but our initial results indicate that PRP treatment of equine tendon and ligament injury is a tremendous improvement over other current techniques.

Research on PRP in horses

Researchers at the University of Barcelona have reported on the use of PRP in tendon and ligament injuries.¹⁰ Ten limbs of 7 horses with tendonitis or desmitis were treated with PRP. Lameness and flexion test scores improved after PRP injection in 6 of 6 horses evaluated. Ultrasound scores improved in 5 of 7 horses. Some horses returned to work as soon as 2 months after PRP injection. One horse with 12 months duration of suspensory desmitis was still improving slowly 9 months after injection of PRP.

Another study of PRP examined gene expression patterns, DNA, and collagen content of equine superficial flexor tendon (SDFT) explants cultured in media consisting of PRP and other blood products. Blood and bone marrow aspirate (BMA) were collected from horses and processed to obtain plasma, PRP, and platelet poor plasma (PPP). IGF-I, TGF-beta1, and PDGF-BB were quantified in all blood products. Tendons were cultured in explant fashion with blood, plasma, PRP, PPP, or BMA at concentrations of 100%, 50%, or 10% in serum-free DMEM with amino acids. TGF-beta1 and PDGF-BB concentrations were higher in PRP compared to all other blood products tested. Tendons cultured in 100% PRP showed enhanced gene expression of the matrix molecules COL1A1, COL3A1, and COMP with no concomitant increase in the catabolic molecules MMP-3 and MMP-13. These findings support in further investigation of PRP as an autogenous, patient-side treatment for equine tendonitis.¹¹

A study of Standardbred racehorses with mid-body suspensory injuries followed 9 horses treated with PRP and 9 age-matched normal racehorses for 3 years. All PRP-treated horses returned to racing in an average of 36 weeks after treatment and 56% of the horses were still racing 3 years later. Total earnings for the two groups did not differ over the three year period. The conclusions of the study were that PRP injection is appropriate and safe for treatment of suspensory desmitis and can result in a productive race career in horses that suffer a suspensory ligament injury.¹²

References

1. Moulin V. Growth factors in skin wound healing. *Eur J Cell Biol* 1995;68:1-7.
2. Zechner W, Tangl S, Tepper G, et al. Influence of Platelet-rich Plasma on Osseous Healing of Dental Implants: A Histologic and Histomorphometric Study in Minipigs. *The International Journal of Oral & Maxillofacial Implants* 2003;18:15-22.

3. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 2003;83:835-70.
4. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and functions. *Platelets* 2001;12:261-73.
5. Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:638-46.
6. Kim S, Chung C, Kim Y, et al. Use of particulate dentin-plaster of Paris combination with/without platelet-rich plasma in the treatment of bone defects around implants. *International Journal of Oral and Maxillofacial Implants*. 2002;17:86-94.
7. Hom DB, Thatcher G, Tibesar R. Growth Factor Therapy to Improve Soft Tissue Healing. *Facial Plastic Surgery* 2002;18:42-52.
8. Carter CA, Jolly DG, Worden CE, et al. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. *Experimental and Molecular Pathology* 2003;74:244-255.
9. Sutter WW, Kaneps AJ, Bertone AL. Comparison of hematologic values and transforming growth factor- β and insulin-like growth factor concentrations in platelet concentrates obtained by use of buffy coat and apheresis methods from equine blood. *Am J Vet Res* 2004;65(7):924-930.
10. Arguelles D, Carmona J, Climent F, Prades M. Clinical experiences with platelet-rich plasma as a treatment of tendon and ligament injuries in the horse. *Proceedings, European College of Veterinary Surgeons*; 2005;217-222.
11. Schnabel LV, Mohammed HO, Miller BJ, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. *J Orthop Res* 2007 Feb;25(2):230-40.
12. Weselau M, Sutter WW, Genovese RL, Bertone AL. Intralesional injection of platelet-rich plasma for mid-body suspensory ligament desmitis in Standardbred race horses. *J Am Vet Med Assoc*. 2008 May 15;232(10):1515-20.