Mechanical Effect of Exogenous Crosslinking on Equine Tendons with Chemically Induced Core Lesions

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Introduction

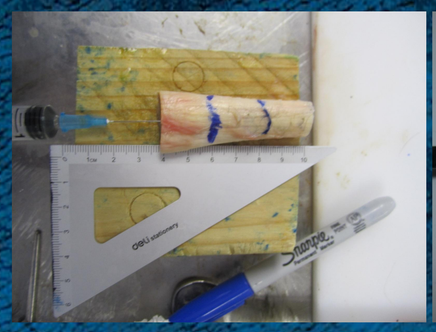
Prior research has shown genipin-based exogenous collagen crosslinking can increase tensile strength, modulus, toughness and permeability of the annulus fibrosis[1,2], plus decrease spinal joint instability by a factor of four[3] including a reduction in instability post-discectomy[4]. Additionally, a recent study demonstrated that exogenous crosslinking can improve mechanical properties of injured equine superficial digital flexor tendons (SDFT)[5]. In that study, injury was simulated by punching a hole in a section of tendon followed by soaking the specimen in a 20mM genipin solution. However, a tendon lesion is more complex and irregular compared to a punched hole, and clinical delivery of a therapeutic dose of a crosslinking reagent would likely require administration via an injection. Therefore, in tandem with a small in vivo equine study, a method was developed in this study to repeatably injure the SDFT ex vivo (or in vivo) and to reliably deliver crosslinking reagent in a clinically relevant manner. These methods were used to evaluate the effects of genipin crosslinking on injured SDFTs.

Methods

A central or core lesion injury was induced chemically using a collagenase injection to a full cross-sectional segment of SDFT. The lesion was subsequently treated with an injection of a buffered genipin reagent. Tensile loading experiments were directed at: 1) evaluating mechanical effects of collagenase on tendons and verifying its appropriateness in producing a core lesion, and 2) quantifying mechanical effects of treating injured tendons with a clinically viable injection of crosslinking reagent. Fifteen 60 mm long proximal segments were divided into 3 groups: control, 1ml of bacterial collagenase (325 units/ml of PBS) injected, and collagenase injected followed by injection of 400mM genipin reagent. Collagenase injections were made into the central 20mm longitudinally, from the proximal end, using a 17G Tuohy tipped epidural needle.

In other experiments, collagenase half-life in 37°C PBS was determined with and without 1 mM calcium. Half-lifes were found to be 24 hours and 4 hours respectively. Collagenase damage was also assessed visually 15 and 24 hours post-injection using different concentrations of collagenase. There was no visual evidence of ongoing collagenase activity after 15 hours in the tissue or with concentrations below 325 units/ml.

After collagenase injection, segments from all groups were incubated @ 37°C for 15 hours. The segments were then injected transversely at the center with either 1ml of 400mM genipin in buffer or a sham injection of PBS. All segments were then incubated @ 37°C incubator for 5 hours to allow the genipin to diffuse and react, and then divided into 3mm tensile test specimens. A punch was used to neck down the center of each specimen to a nominal thickness of 2mm. Width and thickness measurements of the necked-down region were made using a traditional caliper and a laser micrometer (LK-080 , Keyence Corp., Japan). Due to out of plane deformation (flattening from gravity) caused by collagenase induced tissue damage, a correction factor of +32% was applied to the laser measured thickness of the collagenase-only central specimens. This correction factor was calculated based on caliper measurements of the inordinate out of plane deformation observed during the laser micrometry. The middle of each specimen was wrapped in PBS soaked gauze and then the specimen was incubated @ 37°C for 12 hours to completely dry the ends. Duct tape was placed around the dried ends before clamping on a TestResources system (Biaxial L-series). Cyclic loading, stress-relaxation, and load to failure tests were conducted. Specimens derived from the center of each segment were compared separately and also combined with the outer specimens. The number of specimens differed between groups due to variations in segment size. Yield stress and strain were computed using a 0.5% offset method. Mann-Whitney U nonparametric analysis was used to determine significance of differences.



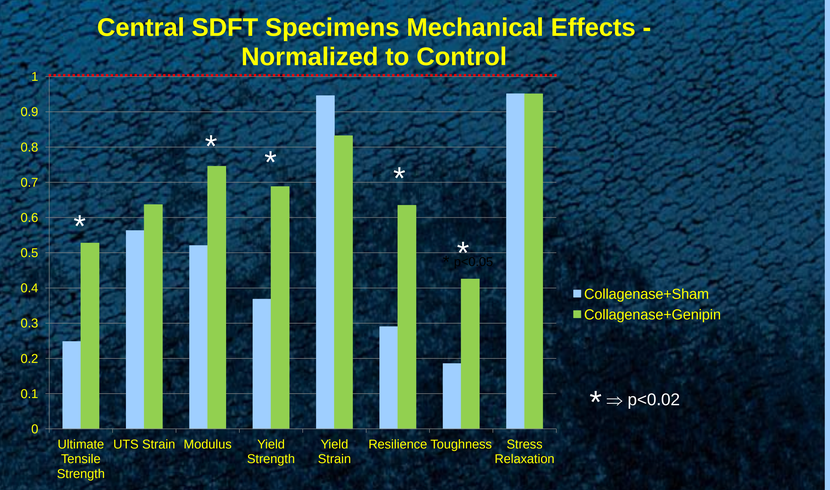
Longitudinal injection of collagenase into central 20mm of SDFT segment.

Transverse injection of genipin (or PBS) into collagenase-induced lesion.

Typical control and genipin treated specimens subjected to tensile mechanical testing.

Results

Injected collagenase reduced ultimate tensile stress by 72.7% ($p<0.001$) in the central specimens. This was accompanied by a 47% reduction in modulus ($p<0.001$), a 78.3% reduction in energy to failure (toughness, $p<0.001$) and a 287.6% increase in cyclic creep (between loading cycles 2 and 10, $p<0.001$). Genipin treated core lesion specimens exhibited a 113% increase in tensile strength ($p=0.007$), 43.2% increase in modulus ($p=0.006$), 129% increase in energy to failure ($p=0.014$) and a 55.6% decrease in cyclic creep ($p<0.001$). Similar effects were observed when both inner and outer specimens were included in the analysis.



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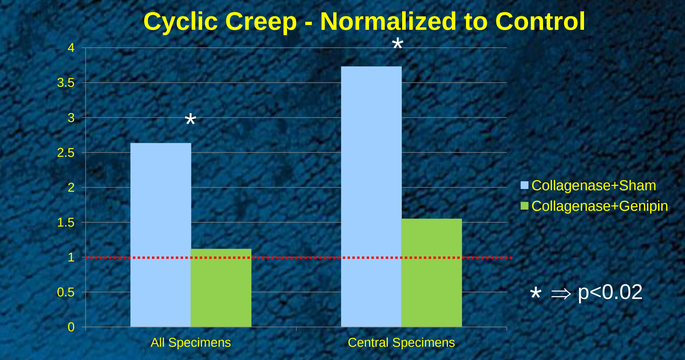
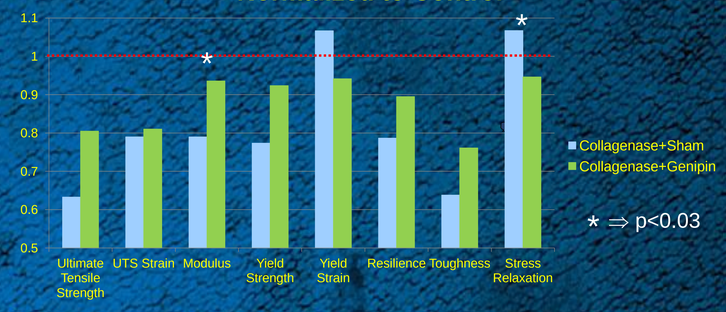
Further financial disclosures available upon request to: thedman@orthopeutics.com





Discussion

Collagenase injection demonstrated a clear mechanical injury effect to the tendon core. Subsequent injection of the genipin reagent showed a marked improvement in the mechanical properties of the injured SDFT with no signs of increased brittleness (ultimate tensile strain was not reduced). Six elastic-plastic and viscoelastic properties of the genipin-treated collagenase-injured core tissue improved between 43% and 130%. However the genipin treated lesions did not improve to normal levels as indicated by control group data.

These data demonstrate that an in-vivo injection of a crosslinking reagent would be capable of providing immediate improvement and strengthening of injured tendon tissue without the need for surgery or biologics. This treatment modality could be practiced alone or together with stem cell therapy which is the current standard of care for equine SDFT injuries. The immediacy of this treatment approach could potentially resist ligament or tendon re-injury during the normal healing process. The risk of tendon re-injury would be a much lesser concern in human tendon injuries compared to the often non-compliant equine patient.

Preliminary in vivo investigations in chemically-induced and actual equine SDFT lesion cases have shown equally favorable results, leading to return to work in half the time required for current (stem cell) treatments. A temporary, mild inflammatory response was observed in all cases, with a reduction in tissue planar waviness and no adverse effects on active tenocytes. Long-term natural repair (filling in) has been observed in the genipin treated tissues.

References:

1.) Chuang et al., Clin Biomech 22(1).
2.) Chuang et al. Spine 35(24).
3.) Hedman et al., Spine 31(15), 2006.
4.) Popovich et al., Spine 36(12).
5.) Fessel et al., J Orthop Res 30(6).