INHIBITING INTERCELLULAR COMMUNICATION BLOCKS WOUND-INDUCED MITOGENESIS IN FLEXOR TENDONS

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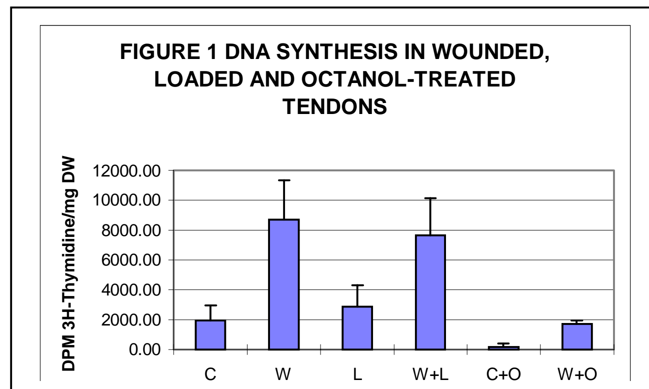
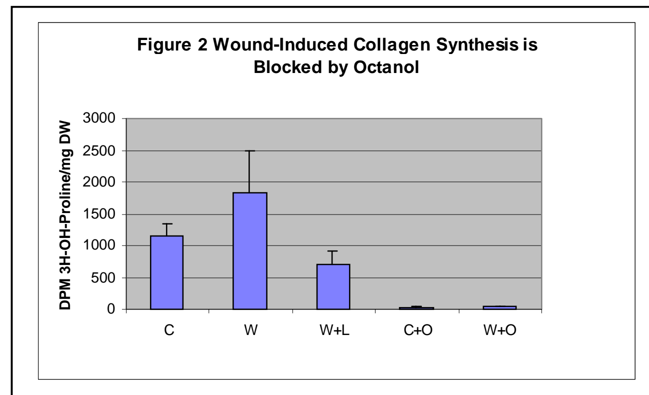
Introduction: Trauma or surgery to a flexor tendon disrupts matrix, blood vessels, nerves and resident cell connections to matrix and each other. Physical therapy post-injury applies directed mechanical loading along the principle strain direction in a tendon. Cyclic motion may assist diffusion of nutrients, growth factors from clot and second messengers. Cells must be able to coordinate their responses to these environmental factors post-wound to achieve a proper healing response resulting in cell division and matrix expression. We hypothesize that cells coordinate their responses by communicating via gap junctions. A gap junction is a group of ion channels through which pass molecules of < 1000MW. They are comprised of hundreds of hemi-ion channels that link to their corresponding halves in neighboring cells to form functional channels and collectively, a gap junction, that are involved in signaling from cell to cell. Mechanically loading a tendon can stimulate cell division and matrix synthesis and result in greater biomechanical strength (1,2). Wounding a tendon also stimulates cell hyperplasia and matrix expression but may result in a weak tendon (3). Motion therapy after flexor tendon surgery results in fewer adhesions and a greater range of motion but may not stimulate cells more than wounding alone immediately post-trauma. We hypothesized that tendon cells must be interconnected and able to signal through gap junctions in order to process mechanical load or growth factor signals during both routine loading, wounding or the combination of treatments.

Methods: Flexor digitorum profundus tendons (FDP, 5 cm long) from the middle claw of 52 day-old White Leghorn chickens were isolated and divided into the following groups (7 tendons/group: 1. clamped in a Tissue Loading Device capable of delivering displacement controlled tension to tendons ex vivo (1 Hz, 2.4% elongation, 8h/day 16h rest, for 3 days), 2. wounded with a double notch wound made by cutting tissue (1 x 25 mm) from each side of the tendon, 3. wounded + 2 mM octanol, 3. wounded and loaded, 4. wounded, loaded, + 2 mM octanol, 5. not wounded or loaded. Each group was incubated in 45 ml of DMEM-H + 10% FCS, 20 mM HEPES pH 7.2, antibiotics and 0.5 mM ascorbate. Samples were labeled with 3H-proline or 3H-thymidine for the last 16 h of the 72h period ex vivo and samples prepared for either DNA synthesis or hydroxyproline analysis. For DNA analyses, tendon samples were aspirated, washed extensively in 5% trichloroacetic acid (TCA) to remove unincorporated radiolabel, dried, weighted and hydrolyzed in 2 N perchloric acid (PCA). Radioactivity in duplicate portions of the PCA supernatant fluid was determined by scintillation counting. Data were expressed as DPM 3H/mg dry weight, per md dry weight. Samples were analyzed for collagen content by washing the samples as above in TCA, drying and weighing the tendons, then hydrolyzing them in 6 N HCl at 106C for 24h. The hydrolyzates were dried, samples reconstituted in dH2O and duplicate portions assayed for hydroxyproline by chloramine T oxidation to the pyrole and extraction into toluene. Radioactivity in hydroxyproline was determined by scintillation counting and data expressed as DPM per mg dry weight.

Results: Data in Figure 1 show that tendons that received a double notch wound had a 4.5 fold increase in DNA synthesis compared to nonwounded controls ($p<0.001$), loaded tendons had a 1.5 fold increase ($p<0.01$), wound + load 3.9 fold increase ($p<0.001$). Treating tendons with a gap junction blocker, 2 mM octanol ablated the wound-induced stimulation, decreasing DNA synthesis to the control level ($p<0.017$). Treatment of control tendons with octanol also reduced DNA synthesis (22% of control, $p<0.004$). To test if octanol is toxic to cells in whole tendon. Tendons were treated with octanol for 24 or 48 h then the octanol washed out with two changes of serum-containing medium, then tendons incubated for remaining time in 10% serum-containing medium. No differences in 3H-thymidine incorporation were detected (Data not shown). Data in Figure 2 indicate that wounding increased collagen synthesis by 1.5 fold compared to nonwounded controls ($p<0.001$). However, wounding and loading decreased collagen synthesis to 62% of the control level ($p<0.001$). Treating tendons with the gap junction inhibitor octanol ablated the stimulatory effect wounding had on collagen synthesis (3% of the control value, 0.001). Collagen synthesis in the control + octanol was also reduced significantly compared to the non-octanol-treated group ($p<0.001$).

Discussion: Tendon repair and the motion therapy strategies are designed to facilitate healing. Hannafin and coworkers showed that cyclic loading of tendons ex vivo at 0.5% elongation for 4 weeks increased the tensile modulus (2). These data indicate that ex vivo loading of tendons increased the biomechanical properties most likely by stimulating first cell division then matrix expression and organization. The best strategy to accomplish the latter would include maximizing cell division in the first few days after load stimulation followed by matrix synthesis. To coordinate these processes, cells need intact gap junctions to communicate signals. We have found that a double notch wound in the avian FDP tendon ex vivo stimulated DNA synthesis more than did a 3 day regimen of mechanical load delivered to tendons ex vivo by a displacement controlled loading device. Both tendon groups were incubated in 10% serum-containing medium because tendons in vitro do not make a significant amount of DNA without adding a growth factor (2). Loading tendons increased collagen synthesis (data not shown) but loading tendons immediately following a double notch wound actually reduced collagen synthesis. These results indicate that application of mechanical load to a tendon immediately following injury may have a positive effect on cell division while having the opposite effect on matrix expression.. Blocking gap junctions in whole tendons with octanol reduced DNA and collagen synthesis in wounded or wounded and loaded tendons to control values. Octanol was not toxic to cells because cells could recover the capacity to synthesize DNA after washing out the drug and releasing the gap junction blockade. We conclude that functional gap junctions are vital to coordinate cells in both DNA synthesis and matrix production post-wound and after mechanical load.





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