HYPERTHERMIA INCREASES THE MAGNITUDE OF CASPASE-3 ACTIVATION AND DNA FRAGMENTATION IN TENDON CELLS UNDERGOING CYCLIC STRAIN: A POTENTIAL INTRINSIC FACTOR IN THE ETIOLOGY OF TENDON OVERUSE INJURIES

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Introduction Repetitive stress (over-use) injuries of tendons are often manifested by localized areas of cell death and an absence of an inflammatory response. Recent studies have suggested that an apoptotic pathway may be responsible for the degenerative pathology seen in chronic overuse injuries (1,2). While *in vitro* studies have documented a magnitude dependent activation of certain stress activated protein kinases (SAPKs) following cyclic strain, this activation was transient and was not able to induce an apoptotic cascade in the cyclically loaded tendon cells (2-4). It has been theorized that local environmental factors such as hyperthermia, osmostic stress, and/or oxidative stress coupled with mechanical stress could activate apoptotic pathways in cyclically loaded cells (4). One study has suggested that heat generated by the release of stored energy in cyclically loaded tendons could increase the local temperature in some areas of the tendon to 44°C (5). It was our hypothesis that cyclic strain in the presence of hyperthermia could increase caspase-3 activation and induce apoptosis (as determined by DNA fragmentation) in tendons cells. Activation of caspase-3 has been implicated in the initiation of apoptosis in several cell lines (6).

Materials and Methods Canine patellar tendon fibroblasts were cultured in DMEM media supplemented with 10% FBS, 0.04M Ascorbate, and 1% antibiotic/antimycotic solution (Gibco, Grand Island, NY). Cells (passage 3) were seeded on to 6-well collagen coated plates (Flexercell® McKeesport, PA) at a concentration of 400,000 cells/well. The plates were divided into the following treatment groups: (a) 37°C; no strain (b) 37°C + cyclic strain @ 9%2hz (c) 40°C; no strain (d) 40°C + strain @ 9%2hz (e) 42°C; no strain (f) 42°C + strain 9%2hz. Each treatment protocol was done for 6 and 24hrs time period. Each group had a minmum of 3 plates per time period. The cyclically loaded cells were subjected to a bi-axial cyclic strain using a computer controlled, pressure driven apparatus (FX-3000 Flexercell® Strain Unit). At the end of the protocol cells were washed with PBS. For DNA fragmentation a Cell Death Detection ELISA kit was used (Roche Diagnostics Corp., Indianapolis, IN) and the enrichmanet factor for each group calculated: Enrichment factor = absorbance of sample/absorbance of negative control. Caspase-3 activity was detected using Caspase-3 assay Kit (Colorimetric) (Sigma, St. Louis, MO) and calculated in μmol p-Nitroaniline released per min per ml. The effect of treatment and time on caspase-3 activity and enrichment factor was evaluated using an ANOVA with a Tukey's post-hoc test. An exponential regression curve was used to correlate DNA fragmentation with caspase-3 activity. Significance was set at $p<0.05$.

Results

Caspase-3 Caspase-3 activity was significantly increased at 42°C in both the 6h and 24h specimens. The addition of cyclic strain significantly increased caspase-3 activity at 42°C after 6h, however it was significantly increased at all temperatures after 24h (Figure 1).

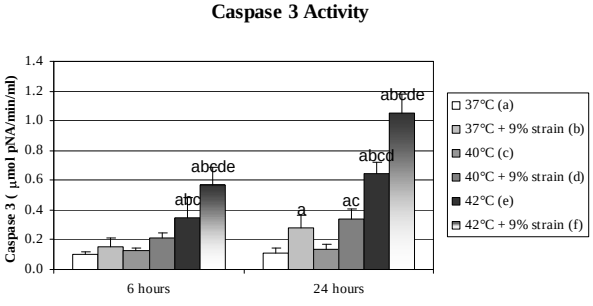

Figure 1 Comparison of different temperatures on Caspase-3 activity at 9% 2hz for 6 and 24hrs (a-e indicates a significant difference between groups at 6/24hrs)

DNA Fragmentation DNA fragmentation was increased at 42°C at 24h. The addition of cyclic strain increased DNA fragmentation at 6h, however it was significantly increased at 24h (Figure 2).

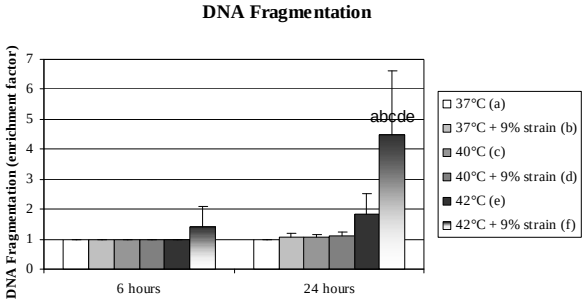

Figure 2 Comparison of different temperatures on DNA fragmentation at 9% 2hz for 6 and 24hrs. (a-e indicates a significant difference between groups at 6/24hrs)

Caspase vs DNA Fragmentation There was a strong ($r^2=0.99$) and significant ($p<0.05$) exponential correlation between Caspase-3 activity and the level of DNA fragmentation (Figure 3).

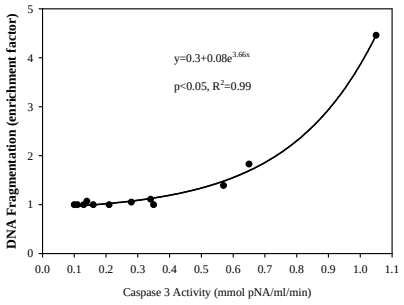

Figure 3 Correlation between DNA fragmentation and Caspase-3 activity with strain at 9%2hz for 6 and 24hrs.

Discussion

Activation of caspase-3 has been implicated as an early event in the apoptotic cascade in a number of cells lines (6). The results of the current study demonstrate that hyperthermia (42°C) alone can cause a significant increase in the activation of caspase 3 in canine tendon cells at both 6 and 24 hrs. However, when cells are cyclically strained for 24 hrs @ 37°C, 40°C, or 42°C caspase-3 activity was significantly increased over non-strained values at the same temperatures. Cyclic strain for 24 hrs in the presence of hyperthermia (42°C) induced a significant rise in the level of DNA fragmentation, a sine qua non of apoptosis (6). The strong exponential correlation between the levels of caspase-3 activation and DNA fragmentation suggests a threshold phenomenon. While lower levels of caspase-3 activation may have little impact on long-term cell survival of tendon cells, once a threshold level of caspase-3 activation is reached an apoptotic cascade is triggered. The results of this study suggest a cumulative effect of cyclic strain and local environmental conditions (i.e. hyperthermia) on the stress response of tendon cells. Such interactions may provide some insight into the intrinsic mechanism(s) which may contribute to the localized areas of cell death seen in repetitive stress (overuse) injuries in tendons.

References

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