MATRIX METABOLISM AND CELL PROLIFERATION IN TENDON -

THE EFFECTS OF NSAIDS ON TENDON REPAIR

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Objectives:
Tendon healing after injury is often a slow process which requires the coordinated control of a number of cell activities including cell recruitment, cell migration, cell proliferation, matrix synthesis and matrix remodeling. In many patients, minor or repeated tendon injury ('overuse') evokes a painful tendinitis which is often chronic and difficult to treat. Chronic tendinitis is thus commonly thought to represent a failure of tendon cells (tenocytes) to repair tendon matrix damage. Non-steroidal anti-inflammatory drugs (NSAIDs), which are commonly used in the treatment of painful tendon conditions, inhibit cyclooxygenases (prostaglandin synthases) and the synthesis of prostaglandins. Some NSAIDs have additonal effects however, including effects on cytokine receptor expression and matrix metabolism. Research on cartilage for example has shown that NSAIDs can be classed into different categories depending on their effect on matrix synthesis - some are inhibitory, some have no effect and some may stimulate syntheis and be 'chondroprotective' In this study we investigated whether there are differences between the cellular activity of tendons from patients with tendinitis compared to normal tendons which would account for the limited repair potential associated with these conditions. We also investigated what effect if any NSAIDs have on tendon matrix metabolism, and whether some NSAIDs have deleterious effects on the ability of tenocytes to repair after injury.

Methods:
Human tendons (n=43) were obtained from patients during orthoapedic, plastic and trauma surgery, including iliopsoas, flexor digitorum superficialis (FDS), flexor digitorum profundus (FDP), supraspinatus, subscapularis and patella tendons. Tendons represented a variety of different pathologies including chronic tendinitis, rheumatoid arthritis, osteoarthritis and trauma. Tendons were dissected into small fragments and replicates (n=6) were cultured in DMEM containing 5% foetal calf serum (FCS). Cell proliferation and proteoglycan synthetic activity of the tendon was measured immediately (day 0) by metabolic incorporation over a 20 hour period using radiolabelled precursors (35SO4 and 3H thymidine) and the response to serum was measured at the end of 7 days (day 7). To investigate the effects of NSAIDs on tendon matrix synthesis, specimens were cultured in medium containing 5% serum and pharmacological concentrations of different NSAIDs (naproxen, diclofenac, indomethacin and aceclofenac). Prostaglandin (PGE_2) was measured in some culture media by RIA. Differences between treatments were assessed for statistical significance using the Students paired T test, with significance at $p<0.05$.

Results:
Normal tendons and tendons from patients with OA had very low rates of synthetic activity at day 0, although higher levels of activity were found in tendons from patients with rheumatoid arthritis and chronic 'tendinitis'.
See Table 1.

Tendon	Proteoglycan synthesis 35S/mg dry wt	
	Day 0	Day 7
Normal (n=6)	262.9	4727.6
OA (n=11)	77.7	806.3
RA (n=10)	686.8	2413.6
Tendinitis (n=5)	4027.9	1768.4

Table 1. Matrix synthetic activity of human tendons.

Metabolic activity was significantly stimulated after 7 days in culture in normal and OA tendons ($p<0.001$), whereas tendinitis specimens showed significantly reduced metabolic activity after culture in 4 out of 6 specimens, with only a small stimulation (ns) of synthetic activity in the remaining 2 specimens.
Addition of NSAIDs to the culture media showed that naproxen and indomethacin were generally inhibitor of tendon matrix synthesis, diclofenac generally had no significant effect and aceclofenac had different effects depending on the nature of the tendon pathology. See Table 2.

Tendon	Naproxen	Diclofenac	Indomethacin	Aceclofenac
Normal (n=6)	82%	100%	71%	160%
OA (n=11)	61%	93%	82%	119%
RA (n=10)	54%	75%	53%	65%
Tendinitis (n=5)	78%	104%	106%	34%

Table 2. Mean percentage change in matrix synthesis after culture with different NSAIDS (day 7)

In patella tendons (n=20), naproxen and indomethacin significantly inhibited proteoglycan synthesis to 54% and 55% of control levels respectively ($p<0.05$), whereas diclofenac and aceclofenac had no significant effect. In tendons from patients with osteoarthritis (n=11), proteoglycan synthesis was reduced to 61% by naproxen and 82% by indomethacin, whereas diclofenac and aceclofenac had no significant effect (93% and 119% respectively) No PGE_2 was detected in NSAID treated culture media, although levels were raised in control cultures (not shown).

Discussion:
Tendon repair involves tendon cell proliferation and new matrix synthesis, and our data is consistent with the hypothesis that some NSAIDs have negative effects cell activity, and should be used with caution in the treatment of tendon injuries. These effects appear to be unrelated to inhibition of prostaglandin synthesis, and may be associated with secondary effects on cytokines or cytokine-receptor expression. The effects of NSAIDs on tendon metabolism similar to the effects reported in cartilage. In most cases, NSAIDs are inhibitory on matrix synthesis, although aceclofenac appears to have differential effects depending on the initial levels of cellular activity in the tendon. Where tendon cell activity is high, as in rheumatoid and tendinitis specimens, the effects of aceclofenac are inhibitory. Where tendon cell activity is initially low, aceclofenac stimulates matrix synthesis. Further work is required to determine the mechanism of this differential response and whether these NSAIDs have the same effects on human tenocyte metabolism *in vivo.*

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