

Influence of neuromodulators and mechanical loading on pathological cell and tissue characteristics in tendinosis

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt försvar i KBC-huset, sal KB.E3.01, fredagen den 10 mars, klockan 13:00.

Avhandlingen kommer att försvaras på engelska.

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Abstract

Background: Tendinosis is a painful chronic, degenerative condition characterized by objective changes in the tissue structure of a tendon. Hallmark features in tendinosis tendons include increased number of cells (hypercellularity), extracellular matrix (ECM) degradation and disorganized collagen. The progression of these pathological changes seen in tendinosis is neither well characterized nor fully understood.

Studies have suggested that there are biochemical and mechanical elements involved in tendinosis. From a biochemical perspective, studies have shown that the tendon cells, tenocytes, produce a number of neuronal signal substances/neuromodulators, such as substance P (SP) and acetylcholine (ACh), traditionally thought to be confined to the nervous system. Furthermore, it has been shown that the expression of these neuromodulators is elevated in tendinosis tendons as compared to normal healthy tendons. Interestingly, studies on other tissue types have revealed that both SP and ACh can induce tissue changes seen in tendinosis, such as hypercellularity and collagen disorganization. From a mechanical angle, it has been suggested that overload of tendons, including extensive strain on the primary tendon cells (tenocytes), causes the degenerative processes associated with tendinosis. *In vivo* studies have shown that in overloaded tendons, the presence of neuromodulators is elevated, not least SP, which also precedes the development of the tissue changes seen in tendinosis. This further supports the importance of combining biochemical factors and mechanical factors in the pathogenesis of tendinosis.

Hypotheses: In this thesis project, we hypothesize: 1) that neuromodulators, such as SP and ACh when stimulating their preferred receptors, the neurokinin 1 (NK-1 R) and muscarinic receptors (mAChRs), respectively, can cause increased tenocyte proliferation; 2) that the effects of SP and ACh on tenocyte proliferation converge mechanistically via a shared signalling pathway; 3) that mechanical loading of tenocytes results in increased production of SP by the tenocytes; and 4) that SP enhances collagen remodelling by tenocytes via NK-1 R.

Model system: *In vitro* studies offer insight into the function of healthy tendon matrix and the etiology of tendinopathy. Using a cell culture model of human primary tendon cells, highly controlled experiments were performed in this thesis project to study a subset of biological and mechanical parameters that are implicated in tendinosis. The FlexCell® Tension System was used to study the influence of mechanical loading on tenocytes. As well, a collagen gel contraction assay was used to examine the intrinsic ability of tenocytes to reorganise type I collagen matrices under the influence of the neuromodulator SP.

Results: The studies showed that exogenous administration of SP and ACh results in increased tenocyte proliferation that is mediated via activation of the ERK1/2 mitogenic pathway when the preferred receptors of SP and ACh, the NK-1 R and mAChRs, respectively, are stimulated. Furthermore, the studies resulted in the novel finding that SP and ACh both converge mechanistically via transforming growth factor (TGF)- β 1 and that a negative feedback mechanism is present in which TGF- β 1 downregulates the expression of mAChRs and NK-1 R. The studies also showed that SP can increase collagen remodelling and upregulate expression of genes related to tendinosis. Finally, it was established that tenocytes are mechanoresponsive by showing that cyclic mechanical loading increases the expression of SP by human tenocytes.

Conclusions: This thesis work concludes that stimulation of NK-1 R and mAChRs results in proliferation of human tenocytes, which both involve the ERK1/2 signalling pathway. It also shows that SP and ACh converge mechanistically via TGF- β 1 in their contribution to tenocyte proliferation. The role of hypercellularity in tendinosis tissue is unknown. Possibly, it has different roles at different stages of the disease. The findings also show that SP increases collagen remodelling, suggesting that increased SP not only results in hypercellularity but also contributes to the collagen morphology in tendinosis.

Keywords

Substance P, acetylcholine, transforming growth factor, neuromodulators, mechanical loading, tendinosis

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