AB0079

EFFECT OF HYALURONIC ACID LOCAL INJECTIONS ON ACHILLES TENDINOPATHIES: AN OBSERVATIONAL STUDY ON TENDON VISCOELASTIC PROPERTIES AND THEIR RELATIONSHIPS WITH CLINICAL OUTCOMES

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Background: Effect of hyaluronic acid local injections on Achilles tendinopathies: an observational study on tendon viscoelastic properties and their relationships with clinical outcomes.

Objectives: Achilles tendinopathy (AT) affects athletes, recreational exercisers and also inactive people, where it is sometimes associated with arthritic phenotypes. The aim of this study was to evaluate the efficacy of a three-local injections regimen of hyaluronic acid (HA) in middle aged patients with a diagnosis of AT; the relationships of the functional, biochemical and clinical outcomes with the viscoelastic properties of the tendon were also studied

Methods: 8 patients previously diagnosed for monolateral AT were enrolled. AT was confirmed before the first local HA injection (T0) by clinical examination, MRI and thermography. At T0 patients were assessed for maximal voluntary isometric contraction (MVI) involving Achilles tendon (both injured and healthy), and pain level with a Likert scale; Achilles tendon viscoelastic state, i.e. tone and stiffness, were then measured at relaxed state and at 10% of MVI with MyotonPro (Myoton Ltd, UK). Finally patients received the first HA injection (RegenFlex T&M, a blend of 2 to 1000 KDa HA, Regenyal, IT). All the measurements were repeated at T1 (15 days after the first injections and immediately prior the second), at T2 (15 days after the second injection and prior the third) and at T3 (15 days after the third injection), i.e. over a total of 45 days in which clinical visits were also performed. Furthermore, before each injection, injured tendon exudates were collected by needle aspiration and the levels of IL-1 β and matrix metalloproteinase 3 (MMP-3) were determined with an ELISA test.

Results: At T0, pain score and MVI were significantly higher and lower in injured tendons, respectively. Accordingly, tone and stiffness values were significantly different between injured and contralateral tendons, especially when measured at the relaxed state. Interestingly, the above differences gradually disappeared at T1, 2 and 3. In keeping with these results, tendon exudates volumes also decreased over time, as well as the levels of IL-1 β and MMP-3.

Conclusion: RegenFlex T&M promoted a progressive healing of AT, with recovery of clinical, functional and tendon's viscoelastic state.

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AB0080

THE INHIBITION OF IL-25 TO THE EFFECT OF IL-17 TO ERK1/2 AND MMP-3 IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIALCYTES

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Background: Rheumatoid arthritis (RA) is a common chronic systemic autoimmune diseases characterized by the inflammation in joint and synovium. Synovitis leads to the destruction of cartilage and bone in joint subsequently joint dysfunction and even disability eventually. Recently, IL-25 has been found to play a role in regulating inflammation through Th1 and Th17 responses in inflammatory diseases^[3]. However, the function to fibroblast-like synoviocytes and its signaling pathway are not clear. This study aims to probe the effects of IL-25 on the expression of ERK1/2 and MMP-3 in RA fibroblast-like synoviocytes.

Objectives: To study the function of IL-25 for rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) differentiation as well as to the effects on the expression of extracellular regulating protein kinase (ERK) and matrix metalloproteinases-3 (MMP-3).

Methods: The differences on ERK1/2 and MMP-3 protein levels were tested in RA-FLS and healthy controls, then IL-17A (10ng/ml), different concentrations of IL-25 (0.01, 0.1, 1 and 10ng/ml) and IL-17A(10ng/ml) to co-stimulate the RA-FLS for 24 hours respectively. The expression of ERK1/2 and MMP-3 protein were detected by the Western blot. The results were expressed by $\pm$ s, t test was used for the comparison between different groups.

Results: The expression of ERK1/2 (1.71 ± 0.17) and MMP-3 (0.50 ± 0.13) proteins in RA-FLS was higher than the healthy controls (0.50 ± 0.15 , 0.17 ± 0.05) ($t=9.13$, $P<0.001$ and $t=4.10$, $P<0.05$), because of the stimulation of IL-17A, the expression of ERK1/2 (0.77 ± 0.22) and MMP-3 (0.59 ± 0.13) proteins in RA-FLS were increased compared with untreated groups (0.18 ± 0.35 , 0.04 ± 0.03) ($t=$

4.69 , $P<0.01$ and $t=-7.47$, $P<0.01$). with the increase of the concentration on IL-25, the level of ERK1/2 (0.54 ± 0.26 , 0.48 ± 0.18 , 0.48 ± 0.23 , 0.23 ± 0.06) and MMP-3 (0.58 ± 0.09 , 0.59 ± 0.14 , 0.21 ± 0.04 , 0.04 ± 0.02) in RA-FLS which stimulated by IL-17A was decreased slowly ($t=4.22$, $P<0.05$ and $t=4.95$, $P<0.01$ and $t=7.47$, $P<0.01$).

Conclusion: IL-25 can inhibit the stimulation of IL-17A on ERK1/2 and MMP-3 fractionally, which imply that it may take part in the development of RA through this pathway and may play as a target for the RA treatment on IL-17A.

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AB0081

NOVEL COOPERATION BETWEEN CCL26 AND CX3CL1 VIA CX3CR1 IN THE INJURY OF SMALL BILE DUCT IN PRIMARY BILIARY CIRRHOSIS

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Background: CX3CL1-CX3CR1 pathway has been found to be critically involved in the pathogenesis of primary biliary cholangitis (PBC)¹. As a novel ligand to CX3CR1, CCL26 can promote chemotaxis of CX3CR1+ immune cells but its role in PBC remains elusive².

Objectives: This study aimed to explore the role of CCL26, together with CX3CL1-CX3CR1 pathway, in the pathogenesis of PBC.

Methods: We recruited 40 patients diagnosed with PBC in the Peking Union Medical College Hospital from Jan 2018 to May 2018 and 18 age and sex-matched healthy controls (HCs). Peripheral blood and liver tissue samples were collected. The plasma level of CX3CL1 and CCL26 were determined by ELISA. Flow cytometry was used to measure the percentage of CX3CR1+ cells in various subsets of PBMCs. The expression of CX3CL1 and CCL26 in liver tissues was revealed by immunohistochemical staining. Using ELISA and flow cytometry, the change of CX3CL1/CCL26-CX3CR1 pathway expression in human intrahepatic biliary epithelial cells (HIBECs) upon stimulation of various cytokines was studied.

Results: The plasma level of CX3CL1 and CCL26 was higher in patients with PBC than HCs with a P value of 0.044 and 0.104 respectively. The increased level of CCL26 was positively correlated with peripheral eosinophils, basophils and CRP level. In comparison with HCs, the expression of CX3CR1 was significantly higher in NKT-like and CD4+ T cells in PBC patients. In liver samples from PBC patients, CX3CL1 and CCL26 were significantly over-expressed in intrahepatic bile ducts and CCL26 also tended to be abundant in hepatocytes near portal areas and gradually weakened in distant regions. This distribution pattern was not observed in HCs. Upon stimulation of IFN- γ , the expression of CX3CR1 on HIBEC surface and CX3CL1 in culture supernatant was significantly up-regulated, while the expression of CCL26 was increased upon IL-4 and IL-13 stimulation.

Conclusion: CCL26 may cooperate with CX3CL1 to mediate the immune injury of intrahepatic bile ducts via CX3CR1 in PBC.

Figure 1: Plasma level of CX3CL1 and CCL26 and expression of CX3CR1 in various subsets of PBMCs. In comparison with HCs, patients with PBC present with following features. (A) A significantly higher plasma level of CX3CL1. (B) An increased tendency of CCL26 level in plasma. (C) A significantly larger percentage of CX3CR1⁺ cells in CD4⁺ T cells. (D) A comparable expression of CX3CR1 in CD8⁺ T cells. (E) A comparable expression of CX3CR1 in NK cells. (F) A significantly larger percentage of CX3CR1⁺ cells in NKT-like cells.

Figure 2: Expression of CX3CL1 and CCL26 in liver tissues and the change of CX3CL1/CCL26-CX3CR1 pathway expression in HIBECs upon stimulation of various cytokines. (A-D) In intrahepatic bile ducts, high expression of CX3CL1 and CCL26, which was indicated by arrows and triangles respectively, was observed in PBC patients. However, only weak expression of CX3CL1 and no expression of CCL26 were found in HCs. (E-F) Weak and no expression of CX3CL1 was revealed in hepatocytes in PBC patients and HCs respectively. (G-H) CCL26 was widely expressed in both PBC patients and HCs. However, in PBC patients, hepatocytes expressed with CCL26 tended to be abundant near portal areas and became scarce in distant regions. This distribution pattern was not observed in HCs. (I) IFN- γ up-regulated the expression of CX3CL1 in HIBEC culture supernatant. (J) IL-4 and IL-13 stimulated the expression of CCL26 in HIBEC culture supernatant. (K) IFN- γ stimulated the expression of CX3CR1 on HIBEC surface.

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AB0082

REDUCED STEROIDOGENIC ACTIVITY OF REPOSITORY CORTICOTROPIN INJECTION (RCI) INDUCES A DISTINCT CYTOKINE RESPONSE FOLLOWING T CELL ACTIVATION IN VIVO

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Background: Melanocortin receptor agonists, such as α MSH have been shown to reduce inflammation in preclinical models, suppressing the production of pro-inflammatory cytokines. Natural and synthetic ACTH analogues remain linked to the induction of corticosteroids as their primary mechanism of action, leading to the anti-inflammatory and immunosuppressive responses. Repository Corticotropin Injection (RCI) is a complex mixture containing purified porcine pituitary ACTH-analogue, and is an FDA-approved treatment for several inflammatory diseases. RCI has been shown to be effective in steroid-refractory autoimmune disease.

Objectives: We hypothesize that Acthar has an immune regulatory response distinct from synthetic ACTH and steroids. These studies sought to explore the differences between RCI and synthetic ACTH on corticosterone levels in rats and cytokine production in a murine T cell activation model.

Methods: RCI (10, 40, or 400 IU/kg) or ACTH (0.6, 1.2, or 2.4 mg/kg) was administered to Sprague Dawley rats, plasma samples collected and analyzed for corticosterone. To determine the effect on T cell cytokine production, Balb/C mice were treated with RCI (10, 40, and 400 U/kg), ACTH (0.6, 1.2, or 2.4 mg/kg) or prednisolone 1 hour prior to the administration of an anti-CD3. Two hours after antibody administration, plasma cytokines were measured using Meso Scale multiplex ELISA.

Results: RCI and ACTH peak corticosterone and area under the curve (AUC) were evaluated. RCI-induced corticosterone rapidly peaked between 2-8 hrs. and was dependent on the dose. ACTH displays a delay in the peak time (8-24 hours) and was not dose dependent. When compared to the high dose ACTH, RCI reduced the AUC by 50.0 $\pm$ 2.6%, 45.5 $\pm$ 5.6% and 27.3 $\pm$ 12.5% respectively for 10, 40, and 400 IU/kg. However, there was no significant reduction between any of the ACTH doses tested.

To further explore functional differences between RCI and ACTH, we examined the effects on T cell activation. In vivo T cell activation with the anti-CD3 antibody (clone, 145-2C11) increased several cytokines, including IL-2, IL-6, IL-10, and IFN γ . Treatment with RCI, ACTH, or prednisolone significantly reduced the production of IL-2, IL-4, IFN γ , and TNF α . Interestingly, RCI showed an inverse dose-response on the production of IL-6 and IL-10. RCI inhibited the production of IL-6 at 74.7 $\pm$ 4.4% and 67.9 $\pm$ 8.5% and IL-10 at 39.1 $\pm$ 7.1% and 26.7 $\pm$ 9.3% for 10 and 40 IU/kg, respectively, whereas 400 IU/kg had no effect. ACTH inhibited the production of IL-6 at a similar level for all doses tested and had no effect on IL-10 production, whereas treatment with prednisolone inhibited all cytokines.

Conclusion: These data show that RCI has a reduced steroidal response compare to synthetic ACTH. RCI has a direct and distinct immunomodulatory response on T cells unique from both synthetic ACTH and steroids. These characteristic effects suggest a mechanism of action for RCI that is steroid-independent and may help explain its benefit in steroid refractory syndromes.

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AB0083

ADIPONECTIN STIMULATES PRO-INFLAMMATORY CYTOKINE PRODUCTION BY PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH EARLY UNTREATED RHEUMATOID ARTHRITIS

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Background: The pathogenesis of rheumatoid arthritis (RA) involves the action of immune cells and fibroblast-like synoviocytes (FLS). Adiponectin, an adipokine produced mainly by adipocytes, is elevated in serum of RA patients compared to healthy controls; moreover, synovial fluid from RA subjects has higher levels of adiponectin compared to controls with osteoarthritis^{1,2}. Adiponectin induces the production of pro-inflammatory cytokines, such as interleukin 6 (IL-6) and IL-8, by