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THE NEUROHISTOLOGY OFPAINFUL AND PAIN-FREE ROTATOR CUFF TENDONS: A CASE CONTROL STUDY

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Purpose: Our main purpose was to compare the tendon neurohistology in two groups of patients; one with significant pain prior to undergoing SAD and one with resolved pain over 6 years post SAD.
Methods: Supraspinatus tendon specimens were obtained using an ultrasound guided biopsy technique from 9 patients with painful RCT resistant to conservative management (painful group) and 9 pain-free patients at over 6 years (median 6 years 11 months) following SAD (pain-free group). Pain symptoms were measured using the validated Oxford Shoulder Score (OSS). Structural tendon integrity was assessed ultrasonographically. The tendon tissue was analysed using basic histological techniques (Haematoxylin and Eosin, and Alcian Blue) and Immunohistochemistry. Image analysis was performed by two blinded observers using Image-J to quantify the amount of 3,3'-diaminobenzidine staining present. Isotype controls were also processed. Mann-Whitney U tests were carried out using SPSS with significance levels set at a minimum of $p < 0.05$.
Results: The groups were similar in terms of age, sex and structural tendon abnormality. The painful group consisted of 7 males and 2 females, the pain-free group of 6 males and 3 females. The mean age of the painful group was 50 years (range 38 to 61) and that of the pain-free group was 53 years (range 39 to 65). The median OSS in the painful group was 32 (range 23 to 34) and this was significantly lower ($p = 0.0002$) than the median OSS in the pain-free group (all 48). There were two partial thickness tears in both groups and no full thickness tears. The modified Bonar scores in the painful and the pain-free groups were comparable. There were no significant differences between groups in terms of cellularity, vascularity, proliferation and hypoxia inducible-factor 1α expression. The leucocyte count (CD45 positive cells) and macrophage count (CD68 positive cells) were increased in the painful group versus pain-free ($p = 0.01$ and 0.05 respectively). The expression of the metabotropic glutamate receptor 7 (mGluR7) was reduced in the painful group versus pain-free ($p = 0.008$ and 0.002 respectively). PGP 9.5 (a nerve marker) expression was increased in the painful group versus pain-free ($p = 0.008$). There were no significant differences in glutamate, the inotropic glutamate receptor (NMDAR1) and the metabotropic glutamate receptors (mGluR1, 2 and 5) between groups.
Conclusions: This study has shown that specific characteristics of tendon histology are associated with a resolution of shoulder pain over six years following SAD. This provides strong evidence that the rotator cuff tendon is of key importance in the symptomatology of RCT. The mechanism behind these tendon differences remains unclear. These findings are novel and improve our understanding of pain in RCT, and may help provide novel therapeutic targets.

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NEGATIVE CORRELATION BETWEEN THE POPULATION OF CD105 POSITIVE SUBSET OF MONOCYTES/MACROPHAGES IN SYNOVIAL FLUID AND THE SEVERITY OF JOINT PAIN AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION SURGERY

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Purpose: Synovial fluid is an interstitial fluid secreted by fibroblastic cells in the synovial membrane. The physiological functions of synovial fluid include reduction of friction, shock absorption, nutrient, and waste transportation in the joint. In addition, previous studies showed that mesenchymal stem cells (MSCs), those are considered to contribute the tissue regeneration, reside in synovial fluid and the number of these cells increased after joint injury. These data strongly suggest that both the contexts of cytokines and cellular components in synovial fluid may greatly influence the recovery process after joint injury or surgery.
In this study, we aimed to analyze the dynamic changes of cellular components in synovial fluid after anterior cruciate ligament reconstruction surgery (ACL-R) and compared them with the clinical conditions such as joint pain of each patient. Here we report that the population of CD105+ subset of Monocytes/Macrophages negatively correlates with the severity of joint pain in the early stage of recovery process after ACL-R.
Methods: This study was approved by the Ethics Committee of Tokyo Medical and Dental University. All patients enrolled in this study, who underwent ACL-R from March till July 2013 in our university hospital, gave their full, written, informed consent for participation prior to the operative procedure (16 cases, Male:11, Female:5, 13-44 year-old, Median:21.5 year-old). Synovial fluid was obtained at day 4 to 5 after surgery and cellular components were analyzed by flow-cytometry (BD FACS Verse™). To evaluate the severity of joint pain semi-quantitatively, we collected Numerical Rating Scale (NRS) Visual Analog Scale (VAS) of each patient at day 4 to 5 after surgery (severity of joint pain when the patient woke up). Pearson's correlation coefficient test was employed for the statistical analysis and values of $p < 0.05$ were considered significant.
Results: Flow-cytometric analyses detected CD3+ T cells, CD56+ Natural Killer (NK) cells, CD66b+ Granulocytes, CD11b+CD14+ Monocytes/Macrophages, and CD44+CD73+CD90+CD105+ MSCs in the synovial fluid at day 4 to 5 after ACL-R, although the population of each cell varied in patients. Population of CD19+ B cells was almost undetectable in all the patients analyzed. Pearson's correlation coefficient test revealed that VAS was negatively correlated with the population of CD105+ cells in synovial fluid ($r = -0.52$, $p < 0.05$). Since we did not observe significant negative correlation between VAS and the population of CD44+CD73+CD90+CD105+ cells, those are less than 10% of total CD105+ cells, we speculated that the CD105+ cells those do not have characteristics of MSCs may have roles in joint pain. Further analyses indicated that CD105+ cells did not co-express CD3, CD56, and CD66b. However more than 90% of CD105+ cells co-expressed CD11b and CD14, suggesting that these cells are a subset of Monocyte/Macrophages. Positive rate of CD105 in total CD11b+CD14+ cells was almost 10%.
Conclusions: Here we showed that the population of CD105+ subset of Monocytes/Macrophages was negatively correlated with the severity of joint pain after ACL-R. We expect that the functional analyses of these cells may give us information to understand the molecular mechanism of joint pain.

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ALTERATIONS IN CENTRAL PAIN PROCESSING ARE NOT RESTRICTED TO END STAGE OSTEOARTHRITIS IN THE MONOSODIUM IODOACETATE MODEL

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Purpose: Osteoarthritis is a group of conditions that causes chronic pain and disability. Mechanisms of central sensitization contribute to the manifestation of aberrant pain responses such as mechanical allodynia and spread of pain beyond the area of tissue damage. Although evidence for central facilitation of pain has been noted in patients undergoing joint replacement for OA, its contribution to early, less severe OA is uncertain. OA severity can be evaluated by the macroscopic appearance of articular surfaces, or microscopic changes in tissue sections. It remains unclear which pathological features mediate OA pain, and therefore the definition of structural disease severity is problematic. The aim of this study was to model OA of differing structural severity by intra-articular injections of low (0.1 mg) and standard doses (1 mg) of monosodium-iodoacetate (MIA) in the rat and to investigate associations between structural features of OA and pain responses.
Methods: Male Sprague-Dawley rats ($n = 8$/group, 330-450g) were anaesthetised and given a single intra-articular injection of 50 μl MIA (0.1 mg or 1 mg) or saline. Pain behaviour was assessed as difference in hind limb weight bearing (%) and mechanical allodynia (hind paw

withdrawal threshold (PWT: g) using calibrated Von Frey hairs) at 0, 3, 7, 9, 14, 16 and 20 days post-injection. Alterations in knee joint structure (cartilage and synovium) were examined by macroscopic visualisation of articular surfaces (Guingamp classification), histology or immunohistochemistry. Differences between groups were analysed using Kruskal Wallis test followed by post hoc Dunn's test. Data are presented as mean (95% confidence interval). Data for correlations between pain and pathology were obtained using pain behaviour scores observed at day of sacrifice. Data are presented as Spearman's correlation coefficients.

Results:

	Saline	0.1 mg MIA	1 mg MIA	Associations between pain and pathology	
WB	PWT				
Pain behaviour					
Weight bearing difference (%)	49.8 (14-85.5)	52.8 (4.78-101)	**189 (118-261)*** ++**	n/a	**-0.44**§
PWT (g)	282 (265-298)	**187 (115-259)***	**187 (160-215)****	**-0.44**§	n/a
Inflammation					
Synovitis	0.7 (-0.2-1.6)	1.6 (0.9-2.4)	**2.5 (2.1-2.9)****	0.27	-0.39
Macrophage area (%)	7.1 (2.9-11)	7.8 (5.1-11)	**16 (11-22)* +**	0.34	-0.21
Joint pathology					
Macroscopic chondropathy	3.4 (1.8-5)	**9.2 (7.4-11)****	**11 (8.4-13)*****	**0.44**§§	-0.28
Cartilage damage	0.5 (0.2-0.9)	**2.2 (1.6-2.9)****	**3.6 (1.3-5.9)*****	0.31	**-0.51**§
Chondrocyte morphology	0.4 (0.3-0.6)	**2.1 (1.5-2.6)****	**2.3 (1.9-2.6)****	0.15	**-0.52**§
Proteoglycan loss (Safranin-O-fast green)	2.0 [1.8-2.2)	**2.4 (2.0-2.8)***	**2.7 (2.4-2.9)****	0.07	-0.37
Proteoglycan loss (Alcian blue-PAS)	0.6 (0.4-0.8)	0.7 (0.38-1)	**1.4 (1.1-1.8)***** ++**	0.15	0.07

Intra-articular injection of MIA produced marked changes in pain behaviour (increase in % weight bearing difference between left and right hind limb and decrease in PWT) and pathology. MIA-induced changes in pain behaviour correlated with changes in macroscopic and microscopic pathology.
$^{***}p < 0.001$ compared to saline control, **++**$p < 0.01$ compared to 0.1 mg MIA and $^{*}p < 0.05$ compared to saline control.

Conclusions: Structural pathology and pain behaviour were dependent on the dose of MIA used. Pain behaviour was associated with severity of structural pathology. The 0.1 mg dose of MIA produced less severity in both pathology and pain behaviour. Reduced mechanical PWT indicating allodynia, in the absence of pronounced weight bearing asymmetry may suggest that mechanisms other than ongoing nociceptive input mediate distal allodynia in the low dose MIA model. Our results show that central augmentation of pain is not restricted to late, structurally severe OA following MIA injection in rats.

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THE EFFECT OF RESINIFERATOXIN PRETREATMENT ON PAIN MEASURED BY DYNAMIC WEIGHT BEARING AND EVOKED PAIN RESPONSES IN AN ACUTE INFLAMMATORY MURINE ARTHRITIS MODEL

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Purpose: Activation of vanilloid receptors by intra-articular (IA) Capsaicin (CAP) injection normalizes Evoked Pain Scores (EPS) and Dynamic Weight Bearing (DWB) measures in carrageenan-induced acute inflammatory arthritis. Resiniferatoxin (RTX) is an ultrapotent CAP analogue that has a similar mechanism of action, and may have greater efficacy on carrageenan-induced arthritis when administered intra-articularly. We hypothesized that mice with acute arthritis would have measurable changes in DWB and EPS due to joint pain, and that these changes could be prevented by pre-treating with IA RTX.

Methods: Acute inflammatory arthritis was produced in C57Bl6mice by IA injection of 10 μl of 2.5% carrageenan into the left knee 3 hours prior to pain behavior testing. Two groups of mice were injected with different doses of IA RTX (10 μl of 0.001% and 10 μl of 0.0003%) 7 days prior to induction of arthritis. Similarly, another group of mice was injected with 10 μl of 0.01% IA CAP 7 days prior to induction of arthritis. DWB was measured with a Dynamic Weight Bearing apparatus (Bioseb, Vitrolles, France). Evoked pain behavior was measured by tallying fights + vocalizations/1 min with repeated firm palpation of the knee.

Results: Increased EPS and reduced DWB measures were clear and reproducible indicators of pain in the affected limbs of arthritic mice. Naïve mice demonstrated low EPS scores (1.01) and equal left to right DWB ratios for weight (1.01) and time (1.00). Induction of acute arthritis by IA Carrageenan resulted in a significantly increased EPS (6.25) and a significant decrease in left to right DWB ratios for weight (0.64) and time (0.89) when compared with controls. Pretreatment with IA CAP 7 days prior to IA Carrageenan resulted in significant improvement in EPS (3.25) and near normalization of left to right DWB ratios for weight (0.975) and time (1.00). Pretreatment with the high dose and the low dose IA RTX 7 days prior to IA Carrageenan lead to significantly improved EPS (1.5 & 1.5, respectively) and left to right DWB ratios for weight (0.85 & 0.82, respectively) and time (0.99 & 0.96, respectively) when compared with the acute arthritis model. IA administration of CAP alone and RTX alone did not have a significant impact on EPS or DWB ratios after 7 days. Immunohistochemical (IHC) evaluation of substance P in the spinal cord dorsal horn demonstrated some differences between naive nonarthritic mice and both arthritic and RTX treated mice.

Conclusions: DWB and EPS are sensitive and specific for quantitation of pain in murine arthritis models. IA Carrageenan administration resulted in a significant increase in EPS and a significant decrease in DWB measures in the affected limb. IA RTX pretreatment in these mice clearly improved pain measures as assessed by EPS and DWB measures and these results were comparable to those previously reported for IA CAP. IA Carrageenan and IA RTX seem to have an enhancing effect on substance P in the dorsal horn as determined by IHC. The potential advantages RTX may have over CAP include a possibly lower therapeutic dose and longer duration of effect. These factors represent important directions for future studies.