Objectives: We undertook this study to explore the roles of DP1 in the development of OA and to evaluate the efficacy of a DP1 selective agonist in the treatment of OA.
Methods: We compared the development of aging-associated OA and destabilization of the medial meniscus (DMM)-induced OA in DP1-deficient (DP1-/-) and wild-type (WT) mice. The progression of OA was assessed by histology, immunohistochemistry, and microcomputed tomography (micro-CT). Cartilage explants from DP1-/- and WT mice were treated with interleukin-1α (IL-1α) ex vivo, to evaluate proteoglycan degradation. The effect of intra-peritoneal administration of the DP1 selective agonist BW245C on OA progression was evaluated in WT mice.
Results: Compared to WT mice, DP1-/- mice had exacerbated cartilage degradation in both models of OA and this was associated with increased expression of MMP-13, and ADAMTS-5. In addition, DP1-/- mice demonstrated enhanced subchondral bone changes. Cartilage explants from DP1-/- mice showed enhanced proteoglycan degradation following treatment with IL-1α. Intraperitoneal injection of BW245C attenuated the severity of DMM-induced cartilage degradation and bony changes in WT mice.
Conclusions: These findings indicate a critical role for DP1 signaling in OA pathogenesis. Modulation of DP1 functions may constitute a potential therapeutic target for the development of novel OA treatments.
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OP0182 CONVERGENCE OF JOINT REPAIR AND PAIN PATHWAYS VIA NERVE GROWTH FACTOR AND P75 EXPRESSING MESENCHYMAL STEM CELLS- A NOVEL EXPLANATION FOR OSTEOARTHRITIS PROGRESSION WITH ANTI-NGF IN OSTEOARTHRITIS

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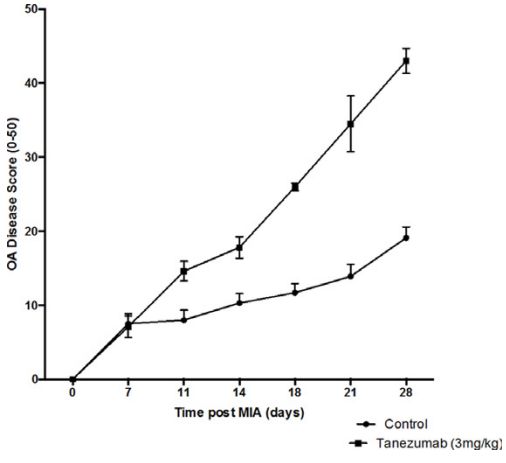
Background: Nerve growth factor (NGF) is a key regulator of pain and anti-NGF therapy reduces osteoarthritis (OA) associated pain. However, anti-NGF therapy is associated with rapidly progressive OA (RPOA) [1]. In hip OA there is a 5-fold increase in mesenchymal stem cells (MSCs) from MRI bone marrow (BM) lesions, areas associated with OA progression [2]. MSCs in such lesions are uniformly positive for the NGF receptor, p75, which is also linked to chemotaxis and proliferation in other stromal cell compartments [3].
Objectives: Evaluate anti-NGF treatment in monoiodoacetate (MIA) induced OA and test whether NGF influences human BM-MSC function.
Methods: Human tibial plateau (TP) bone was isolated from patients undergoing total knee replacement. OA was induced in male Wistar rats (n=6) by intra-articular injection of 0.3 mg MIA. Each animal was treated with subcutaneous injection of control human IgG or anti-NGF (3 mg/kg) at Days 5, 10 and 15. Human and animal tissues sections were prepared for histological analysis using H&E staining and immunohistochemistry (IHC) using anti-p75 and anti-NGF antibodies. BM-MSCs were isolated from iliac crest aspirates and cultured under normal conditions. Expression of p75 was induced following overnight incubation with 400mM ethanol (EtOH) and confirmed by flow cytometry. Proliferation was assessed ± EtOH and 0–1 μg/ml NGF.
Results: Regions adjacent to cartilage loss in human TP showed abundant stromal proliferation, NGF and p75 immunoreactivity. In MIA model, by Day 18 there was substantial loss of cartilage, bone remodelling and stromal proliferation mimicking human disease. By Week 4 animals demonstrated unequal weight-bearing ($p<0.05$). Anti-NGF provided sufficient analgesia to normalise weight-bearing. Compared to IgG control, arthropathy progression was faster (Fig) with complete cartilage loss, bone marrow necrosis and cyst formation by Day 21. p75 immunoreactivity was greatly reduced in MIA-injected rats receiving anti-NGF compared with IgG. NGF positive staining was widespread in naïve and MIA-injected knees, but almost absent from MIA-treated knees of anti-NGF treated rats.



To investigate increased p75 positivity in knee OA, we restored p75 expression loss in expanded cell, to 97% of BM-MSCs (n=3) using EtOH. Increased MSC proliferation was seen at Days 6 and 9 (26%, p=0.03 and 30%, p=0.01 increase respectively, n=7) for 1 μg/ml NGF in the presence of EtOH compared to control (no NGF). In cultures without EtOH induction (absent p75) NGF had no effect.
Conclusions: TP bone from OA patients and rat MIA-treated subchondral bone contains p75 positive staining in regions of cartilage destruction and associated NGF positivity. Anti-NGF treatment exacerbated MIA-induced OA and reduced NGF and p75 expression. In vitro, NGF increased BM-MSC proliferation, suggesting NGF may be involved in the stromal proliferation seen at sites of OA damage. Thus, NGF may regulate MSC function. Complete blockade represents a novel mechanism for accelerated joint destruction in OA.
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OP0183 CORDYCEPIN, A NOVEL COMPOUND, REDUCES KNEE JOINT PATHOLOGY AND PAIN IN THE MONOSODIUM IODOACETATE (MIA) RAT MODEL OF OSTEOARTHRITIS

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Background: Cordycepin (3' deoxyadenosine) is a popular traditional medicine in Asia, taken for conditions associated with ageing. Osteoarthritis (OA) is a common cause of pain and disability in the ageing population. Inflammation is a key component of osteoarthritic pain. Cordycepin is thought to act by inhibiting polyadenylation, the last step of mRNA synthesis, and can potentially have increased therapeutic benefit in OA (anti-inflammatory and analgesic) with fewer side effects than currently available therapies.
Objectives: The aim of this study was to determine whether cordycepin treatment alters osteoarthritic pain and pathology, and to decipher the mechanisms of action by which cordycepin exerts any potential beneficial actions.
Methods: OA was induced in male Sprague Dawley rats by intra-articular injection of mono-sodium iodoacetate (MIA; 1mg/50μl) on day 0. Cordycepin was administered orally (2mg/rat mixed in 1g of wet mash) every other day for 2 weeks (pre-emptive study: day 0 to day 14, therapeutic study: day 14 to day 28). Pain behaviour was measured as hind-limb weight-bearing asymmetry and mechanical paw withdrawal thresholds. Joint tissues were collected at days 14 and 28. Joint changes were quantified using histology and immunohistochemistry techniques. Synovial inflammation was quantified as extent of CD68 positive macrophage and cellular infiltration. Synovial angiogenesis was measured as endothelial cells positive for proliferating cell nuclear antigen (PCNA). Safranin-O staining was used to score cartilage damage and bone changes (osteophytes and channels crossing the osteochondral junction [OCJ]). Tartrate-resistant acid phosphatase (TRAP) positive osteoclasts and ADAMTS-5 and MMP13 positive chondrocytes were quantified as additional markers to detect bone and cartilage changes.
Results: The MIA rodent model of OA pain exhibited significant pain behaviour, synovial inflammation and angiogenesis, cartilage damage, osteophyte formation and subchondral bone changes, compared with non-arthritic controls. A two week pre-emptive and therapeutic treatment with cordycepin reduced MIA-induced pain behaviour and synovial changes (inflammation and angiogenesis). Pre-emptive cordycepin treatment reduced cartilage damage and the level of ADAMTS-5 and MMP13 from the chondrocytes. Pre-emptive and therapeutic cordycepin treatment reduced the number of channels crossing the OCJ and TRAP positive osteoclasts in the subchondral bone, but had no effect on numbers of osteophytes. Therapeutic cordycepin treatment did not alter cartilage damage score or the level of ADAMTS-5 and MMP13 positive chondrocytes.
Conclusions: Our data show that the analgesic effects of orally administered cordycepin in a pre-emptive and therapeutic protocol are associated with synovial changes (inflammation and angiogenesis) and bone remodelling. Administration of cordycepin before the onset of MIA-induced OA reduced cartilage damage and had a chondroprotective effect. Whereas therapeutically administered cordycepin did not alter cartilage damage. Further studies will investigate whether cordycepin mediated reduction in MIA-induced pathology and pain behaviour is as a result of its direct action on polyadenylation inhibition. Polyadenylation inhibitors could therefore be a novel class of drugs for treating OA.
Disclosure of Interest: None declared

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OP0184 IMSYC IMMUNOLOGIC SYNOVITIS SCORE: A NEW HISTOLOGICAL SCORE FOR DISCRIMINATING INFLAMMATORY AND NON-INFLAMMATORY ARTHRITIS

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Background: General Synovitis score (GSS) has been developed by Krenn *et al.* in order to discriminate inflammatory arthritis (IA) and non-inflammatory arthritis (NIA) (1). This score assesses 3 major components of synovitis: lining layer hyperplasia, activation of resident cells (stroma) and inflammatory infiltrate. All components are graded semi-quantitatively from 0 to 3 and the total score is on 9. High-grade synovitis is highly associated with IA and is defined by a score upper than 5 with a sensitivity of 61.7% and a specificity of 96.1%. As immunohistochemistry (IHC) is frequently used to better characterize synovitis, we propose to create a new IMmunologic SYnovitis SCore (IMSYC) adding 5 components to the GSS: CD68, CD3, CD20, CD31 and Ki67 immunostaining.
Objectives: Our work aimed to evaluate the diagnostic performance of this new score including IHC, to define the best cut off for inflammatory arthritis recognition, and to compare its diagnostic performance with the GSS.
Methods: 53 synovial samples from patients were obtained during surgery (arthroplasty or synovectomy). All patients gave written consent prior surgery. Samples were cut and Hematoxylin and eosin stained. CD68, CD3, CD20, CD31 and KI67 Immunohistochemistry were performed. GSS was assessed for each slide and semi-quantitative 4 scale scores (0–3) were given for each immunostaining, in a blind manner. The score is calculated on 24 (GSS 0–9 points, and 0–3 score for each of the 5 immunostaining). A representative amount of slides was read by 2 observers with a good interobserver variability (spearman correlation coefficient of 0.95, $p<0.0005$). They then defined a consensual and reproducible scoring atlas.
Results: 53 patients were included. 25 were females (47,2%), mean age was 62.1 years [Standard deviation (SD) 13.2 years]. 36 had inflammatory arthritis reparsed as follows: 28 Rheumatoid arthritis (RA), 5 had Psoriatic arthritis, 3 had Undifferentiated arthritis. “Non inflammatory” arthritis group included 10 patients with Osteoarthritis and 7 with ligaments or meniscus injuries. Mean GSS was significantly higher in the IA group 5.70 [SD 0.321] vs.3.51 [SD 0.351]; $p<0.001$). Mean IMSYC was significantly superior in the IA group 14.94 [SD 0.747] vs. 8.50 [SD 0.639]; $p<0.001$). In univariate analysis by logistic regression, GSS (Odd Ratio (OR) 2.27; $p<0.001$), CD3 (OR 4.3; p=0.002), CD68 (OR 4.5; p=0.002), Ki67 (OR 11.8; $p<0.001$), and CD31 scores (OR 6.5; p=0.001) were significantly associated with IA, however CD20 score was not (OR 0.9; p=0.34). ROC curve analysis determined the score of 10.5 out of 24 as the best cut off for discrimination between IA and non-IA with a sensitivity of 74.3% and specificity of 100%. The area under ROC curves was statistically superior with IMSYC (0.93) compared to GSS (0.81) (p=0.05).
Conclusions: We hereby propose a new synovitis score including IHC. This score has a better sensitivity and specificity than the Global synovitis score for discrimination between IA and non-IA. Moreover, this score accurately describes synovial membrane immunophenotype and could therefore give a basis for tissue driven therapies in rheumatic diseases, especially in RA.
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OP0185 IMMUNOHISTOLOGIC STUDY OF SYNOVITIS FROM PATIENTS WITH UNDIFFERENTIATED ARTHRITIS WHO EVOLVED TO RHEUMATOID ARTRHITIS OR PSORIATIC ARTHRITIS AFTER FOLLOW-UP

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Background: Undifferentiated Arthritis (UA) is defined as an inflammatory oligo/poly arthritis that does not fulfil criteria for a definitive diagnosis.Delay in diagnosis and treatment leads to poor prognosis. Previous studies have found differences in the cellular infiltrate between the synovitis of Rheumatoid Arthritis (RA) and Spondyloarthritis, including psoriatic arthritis (PsA)
Objectives: To identify synovial biomarkers that may be useful to diagnose patients with early UA
Methods: Retrospective longitudinal study.Patients with UA followed in our Arthritis Unit,who underwent arthroscopy between 2000 and 2014.Synovial biopsy were stained by immunohistochemistry with the following antibodies:CD3 for T cells,CD20 for B cells,CD79 for B cells,CD138 for plasma cells,CD31 for vessels,CD68 for macrophages,CD15 for neutrophils,CD117 for mast cells and hsp47 for fibroblasts, and quantified by Digital Image Analysis (Olympus).The same antibodies were evaluated in RA and PsA control groups
Results: 55 UA and 78 controls were included.Table 1 shows the clinical, serological and demographic characteristics.Among patients with UA, 23 (42%) patients met criteria for RA and 32 (58%) for PsA during follow-up.Synovitis of patients with UA had higher macrophage (CD68+) density in total tissue (p=0.008) and sublining (SL) (p=0.012) than the control group.The UA that evolved to RA had a higher density of CD3 T lymphocytes than the control RA group (p=0.014). No differences were observed in cells of adaptive immunity (CD20 B lymphocytes, CD138 plasma cells), innate immunity (CD117 mast cells, CD15 neutrophils), vessels (CD31) between the 4 groups.The area (%) stained by anti-hsp47 (synovial fibroblasts) in SL was higher in the RA control group than in the PsA (p=0.003)

Table 1. Data are expressed as mean±SD

	UA n=55	UA-RA n=23	UA-PsA n=32	p	RA n=40	PsA n=38	p
Age (years)	47±13	51±13	44±12	0.058	60±12	54±13	0.065
Sex (male)n (%)	22 (40)	6 (26)	16 (50)	0.074	17 (43)	23 (61)	0.111
Disease duration (years)	3±4	3±2	3±4	0.114	3±6	2±2	0.851
Time of follow-up (years)	7±4	8±4	6±4	0.038	7±3	5±3	0.008
CRP (mg/dL)	2.7±3.9	2.4±1.9	3.0±4.9	0.189	3.8±3.2	3.0±3.8	0.102
DAS28 (ESR)	4.17±1.05	4,83±1,06	3,63±0,94	0.000	5,15±1,51	4,02±1,04	0.002
ACPA n (%)	4 (22)	4 (22)	0 (0)	0.000	30 (77)	0 (0)	0.000
RF n (%)	10 (7)	9 (41)	1 (3)	0.000	28 (70)	1 (3)	0.000
csDMARD n (%)	21 (41)	7 (35)	14 (45)	0.472	29 (73)	19 (50)	0.041
bDMARD n (%)	2 (4)	0 (0)	2 (6)	0.243	8 (20)	6 (16)	0.628
PDN n (%)	7 (13)	3 (14)	4 (13)	0.851	11 (28)	5 (13)	0.117

Conclusions: This is the first immunohistological study of synovitis in a significant group of patients with UA who developed AR or PsA during follow-up. Although there are some differences between the UA and control groups in the density of CD68+ macrophages and lymphocytes T CD3+, these do not appear to be useful for an early diagnosis of UA. On the other hand, unlike the results of some previous studies, we not found differences between the cellular infiltrate (adaptive immunity, innate immunity or vessels) in patients with RA and PsA. The fact that some patients with UA were undergoing treatment prior to synovial biopsy and its retrospective character limit the results of this study
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OP0186 TENOFOVIR, A NUCLEOSIDE ANALOG REVERSE TRANSCRIPTASE INHIBITOR FOR TREATMENT OF HIV, PROMOTES OSTEOCLAST DIFFERENTIATION AND BONE LOST IN VIVO IN A MECHANISM DEPENDING ON ATP RELEASE AND ADENOSINE, AND DIPYRIDAMOLE MAY BE A USEFUL TREATMENT TO REVERT THE EFFECTS

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Background: HIV infection devastates the immune system but also affects other tissues and organs Bone alterations have been observed in HIV disease for nearly two decades, in particular a higher risk of low bone mineral density (BMD) and fragility fractures. Treatment with Tenofovir alone or as part of HAART, leads to changes in bone catabolism markers and significant reductions in BMD in children and young adults. Tenofovir is taken up by cells and phosphorylatedand inhibits HIV-reverse transcriptase by mimicking AMP. We have recently found that Tenofovir inhibits Pannexin-1/Connexin-43-mediated ATP release from cells and decreases extracellular adenosine levels and fibrosis in murine models. Inhibition of osteoclast formation *via* adenosine A2A receptor stimulation or increasing local adenosine concentration stimulates new bone formation as well as rhBMP-2.
Objectives: As adenosine and ATP are key regulators of bone homeostasis, we determined whether Tenofovir directly affects bone by an adenosine- or ATP-dependent mechanism and if treatment with Dipyridamole, an agent that increases extracellular adenosine by blocking cellular adenosine uptake, may be a useful treatment to counteract Tenofovir effects.
Methods: M-CSF/RANKL-induced osteoclast (OC) was studied in primary murine bone marrow culture as the number of TRAP-positive cells after challenge with Tenofovir (1nM-100μM) alone or in combination with Dipyridamole (1nM-100μM). OC markers were measured by RT-PCR. Pannexin-1 and Connexin-43 expression were permanently knocked down by lentiviral infection with appropriate shRNA or scrambled shRNA and these cells were induced to differentiate into OC by RANKL. Male C57Bl/6 mice received Tenofovir 75mg/Kg/day alone or in combination with Dipyridamole 1mg/Kg/day for 4 weeks. Double labelling of bone with calcein/Alizarin Red to analized bone formation was performed and long bones prepared for microCT and histology.
Results: Tenofovir produced a dose-dependent increase in OC differentia-