Methods: A rat model for RA was used with repeated intra-articular methylated bovine serum albumin (mBSA) injections in one knee ("arthritic" knee), the contralateral knee serving as internal control. Recombinant human AP (200 µg, s.c.) was administered twice (spaced 4 days) before mBSA injections (*prophylactic setting*) or after arthritis induction (4x, 2x/wk, *therapeutic setting*), or combined with MTX (0.3 mg/kg or 1 mg/kg, i.p.) in 4 rats/group. Plasma pharmacokinetics of AP in arthritic and healthy rats was monitored by colorimetric enzymatic assay. As an endpoint of AP/MTX treatment outcome, macrophage infiltration (marking arthritic conditions) in knee sections, liver and spleen was assessed by immunohistochemistry (ED1 and ED2-macrophage specific antibodies), immunofluorescence (macrophage marker; Folate Receptor-β, FRβ), and positron emission tomography (PET) scans and *ex vivo* tissue distribution with the macrophage tracer [¹⁸F] fluoro-PEG-folate targeting FRβ.

Results: After AP administration, both in healthy and arthritic rats, plasma AP levels increased over 1 hour to reach a maximum of 50%–70% above baseline. Increased plasma AP levels in healthy rats were retained for at least 4 hours, whilst in arthritic rats AP plasma levels steadily returned to baseline levels within this time frame, suggesting consumption of available AP by conjugating to its ITM substrates. Prophylactic and therapeutic schedules of AP treatment, either as single agent or in combination with MTX, were well tolerated. Both prophylactic and therapeutic AP markedly reduced synovial macrophage infiltration in arthritic knees (ED1; 3.5–4 fold, ED2; 3.5–6 fold), comparable with MTX treatment effects. AP/MTX combinations slightly improved on single agent effects. PET monitoring and *ex-vivo* tissue distribution studies corroborated the impact of AP, MTX and AP/MTX on reducing synovial macrophage infiltration. Beyond localised articular effects, AP also displayed systemic anti-inflammatory effects by a 2-fold reduction of ED1, ED2 and FRβ-positive macrophages in liver and spleen of arthritic rats.

Conclusions: AP as single agent and combined with MTX elicits local and systemic anti-arthritis activity in arthritic rats and appears promising as a new therapeutic compound against arthritic conditions.

REFERENCE:

- [1] Pike AF, et al. *Biochim Biophys Acta* 2013;1832:2044–56.

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AB0116

ADIPOSE DERIVED STEM CELLSUPPRESSED SYNOVIAL INFLAMMATION IN SKG/JCL MICE IN VIVO AND INFLAMMATORY CYTOKINE IN SYNOVIAL FIBROBLAST IN VITRO

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Background: Adipose derived stem cell (ADSC) is one of the stem cells produced by adipose tissue which can be collected easily and in large quantities. It has been reported the anti-inflammatory effect of ADSC in some disease models.¹ However, the effect of ADSC for synovitis such as rheumatoid arthritis (RA) is unknown.

Objectives: The aim of this study is to investigate the effects of ADSC for joint synovitis and cartilage degeneration in SKG/Jcl mice *in vivo* and the effects of ADSC for synovial fibroblast *in vitro*.

Methods: SKG/Jcl mice which developed auto-immune arthritis by adjuvant stimulation were used as RA animal model.² *In vivo*, the intra-articular injections of ADSC (ADSC group; n=10) or PBS (PBS group, n=10) were performed to the bilateral knee of SKG mice with arthritis. The knee joint was histologically assessed with synovitis score and Mankin score at 2 weeks after ADSC injection. *In vitro*, the anti-inflammatory effect of ADSC for stimulated human synovial fibroblast was analysed by real-time RT-PCR.

Results: *In vivo*, the synovitis score and Mankin score were statistically lower in ADSC group (synovitis score 2.0±0.7 vs 6.0±1.6, p<0.01 and Mankin score 2.2±0.8 vs 4.9±0.8, p<0.01) (figure 1). *In vitro*, the expression of tumour necrosis factor-stimulated gene-6 (TSG-6), the anti-inflammatory cytokine, was significantly higher in ADSC than in synovial cell (p<0.01). The inflammatory cytokine levels in

stimulated synovial cell were significantly decreased by ADSC treatment (p<0.01) (table 1).

Abstract AB0116 – Table 1. Gene expressions in each cell by real-time RT-PCR

	Relative gene expressions referenced GAPDH as 1 (mean ±SD)		
	IL-6	TNF-α	TSG-6
ADSC	0.39±0.03	0.37±0.1	0.77 ±0.06*
Synovial fibroblast	0.21±0.07	0.35±0.1	0.08±0.03
Stimulated synovial fibroblast (non-treatment, 24 hour)	77.8±1.5	38.9±0.1	5.2±0.7
Stimulated synovial fibroblast (non- treatment, 48 hour)	34.9±0.5	39.9±0.1	4.2±0.3
Stimulated synovial fibroblast (ADSC treatment, 48 hour)	5.2 ±0.06**	9.8 ±0.1**	1.9±0.1

ADSC; adipose derived stem cell, IL; interleukin, TNF; tumour necrosis factor, TSG; tumour necrosis factor-stimulated gene.

*p<0.01 (the comparison to synovial cell by Mann-Whitney U test)

**p<0.01 (the comparison to non-treatment group by Mann-Whitney U test)

Abstract AB0116 – Figure 1. The anti-inflammatory effects of intra-articular ADSC injection. The H-E stain of knee joint in SKG/Jcl mice (a) and PBS treated SKG/Jcl mice after induction of synovitis (b). The synovial proliferation was suppressed in ADSC treated SKG/Jcl mice after induction of synovitis (c). The Safranin-O stain in SKG/Jcl mice without synovitis (d), PBS treated SKG/Jcl mice (e) and ADSC treated SKG/Jcl mice (f) after induction of synovitis. The cartilage staining was reduced in SKG/Jcl mice with inflammatory synovitis (yellow arrows).

Conclusions: This is the first report to show the anti-inflammatory effect of ADSC for synovitis in RA animal model. ADSC can be collected with a minimally invasive technique more easily than other mesenchymal stem cells. ADSC might have potential to be one of the RA treatment.

REFERENCES:

- [1] Pers YM, et al. Adipose derived stem cells for regenerative therapy in osteoarticular diseases. *Horm Mol Biol Clin Invest* 2016 Dec 1;28 (3):113–120.
[2] Sakaguchi N, et al. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* 2003;426:454–60.

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AB0117

ASSOCIATION OF VITAMIN D DEFICIENCY WITH CRP/ ACPA POSITIVITY IN ALGERIAN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Vitamin D (VD) is a steroid hormone belonging to the class of secosteroids with myriad immune functions and seems to be involved in the development and severity of rheumatoid arthritis (RA).^{1–3}