Introduction Gout flares are characteristically mediated by the pro-inflammatory cytokine interleukin (IL)-1 β .¹ Uptake of monosodium urate (MSU) crystals by macrophages activates the nucleotide-binding domain and leucine-rich repeat containing family, pyrin domain containing 3 (NLRP3) inflammasome, which converts intracellular pro-interleukin-1 β (pro-IL-1 β) to mature bioactive IL-1 β by proteolytic cleavage. Deposition of MSU crystals alone is not sufficient to trigger a gout flare. In the presence of concomitant pro-inflammatory stimuli, i.e. Toll-Like Receptor (TLR) agonists, transcription of *il-1 β* gene is induced and pro-IL-1 β is rapidly converted into its active form. IL-1 β binds to its receptor (IL-1R1) and induces a cascade of secondary inflammatory mediators including prostaglandins, cytokines and chemokines. Recently, OLT1177TM, a β -sulfonyl nitrile compound, safe in humans, was shown to inhibit the NLRP3 inflammasome, reverse the metabolic cost of inflammation and inhibit joint inflammation in murine models of acute arthritis.^{2,3}

Objectives To explore the mechanism by which oral OLT1177TM inhibits joint inflammation in humans with gout flares.

Methods 29 patients with a gout flare were treated within 4 days after the start of the symptoms with three different doses of OLT1177TM for 7 days (EudraCT: 2016-000943-14). Blood was drawn at baseline, days 3, 7 and 14 (7 days after finishing treatment). Haematology, hsCRP and SAA were measured as markers for systemic inflammation. Plasma was collected for assessment of circulating cytokines. *Peripheral blood mononuclear cells (PBMCs) were isolated and cultured under unstimulated or stimulated conditions with a TLR ligands (Pam3Cys and LPS) in combination with MSU crystals after which intra- and extracellular cytokine production was assessed.*

Results Plasma IL-1 β are increased in samples from intercritical gout patients and individuals with gout flares when compared to healthy controls (healthy controls hsCRP and SAA and acute phase proteins of individuals treated with OLT1177TM during a gout flare show a dose-dependent reduction during course of treatment. Circulating IL-1 β and IL-6, but not TNF α , was reduced and stimulated cytokine production of PBMCs declined during treatment. In unstimulated PBMCs on day 3, ratio of intracellular pro-IL-1 β and IL-1 β revealed inhibition of the NLRP3 inflammasome by oral OLT1177TM. Moreover, *in vitro* and *ex vivo* data show OLT1177TM spontaneously increases the level of IL-1 receptor antagonist (IL-1Ra).

Conclusions Oral OLT1177TM, safe in humans, inhibits inflammation and increases systemic IL-1Ra concentrations in patients with an acute gout flare by inhibiting the NLRP3 inflammasome product, IL-1 β .

REFERENCES

1. Dinarello CA. How interleukin-1 β induces gouty arthritis. *Arthritis Rheum* 2010;**62**(11):3140–4.
2. Marchetti C, et al. OLT1177, a beta-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation. *Proc Natl Acad Sci USA* 2018;**115**(7):E1530–E1539.
3. Marchetti C, et al. NLRP3 inflammasome inhibitor OLT1177 suppresses joint inflammation in murine models of acute arthritis. *Arthritis Res Ther* 2018;**20**(1):169.

Acknowledgements We wish to acknowledge the gout patients who participated as well as Daniëlle Poeth and Dorine Baselmans for their commitment to the conduct of this study.

Potential investigators conflict of interest DS is Founder and CEO of Olatec, CD is CSO and SAB member of Olatec, LJ is SAB member of Olatec.

Disclosure of Interest None declared.

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THE FIRST PHASE 2A PROOF-OF-CONCEPT STUDY OF A SELECTIVE NLRP3 INFLAMMASOME INHIBITOR, DAPANSUTRILETM (OLT1177TM), IN ACUTE GOUT

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10.1136/annrheumdis-2018-EWRR2019.142

Career situation of first and presenting author Young investigator.

Introduction Acute gout is a severe debilitating type of arthritis that is treated in the acute phase with potent anti-inflammatory drugs. To date, prednisolone, colchicine and/or non-steroidal anti-inflammatory drugs are the standard of care despite serious side effects of chronic use, especially in the older population most often presenting with an acute gout attack. In addition, interleukin-1 (IL-1) biologics (e.g., canakinumab/rilonacept/ anakinra) have proven efficacy in RCTs, however, these biologics have not been broadly adopted due to barriers such as the requirement of parenteral administration, cost and risk of infection. There is an unmet need for a safe, oral, cost effective IL-1 inhibitor targeting the NLRP3 inflammasome pathway.

DapansutrineTM (OLT1177TM) has been shown to inhibit IL-1 β and IL-18 release in human macrophages and to prevent NLRP3 inflammasome activation, with no effect on the priming phase of the NLRP3 inflammasome formation or on TNF- α . DapansutrineTM has no effect on the AIM2 or NLRC4 inflammasomes. In addition to acute gout, dapansutrineTM is under clinical development in heart failure and it has demonstrated positive results in numerous preclinical models. Dapansutrine'sTM Phase 1 dose escalation clinical trial demonstrated safety at doses up to 1000 mg/day for 8 days.

Objectives The Phase 2a study is a dose ranging, proof-of-concept trial to demonstrate the clinical effectiveness, pharmacodynamics (e.g., cytokine levels and other relevant biomarkers), safety/tolerability and pharmacokinetics of dapansutrineTM in four cohorts at doses of 100 mg QD, 300 mg QD, 500 mg BID or 500 mg QID.

Methods An adaptive dose design was used with planned enrollment of 8 patients per cohort to assess the efficacy of dapansutrineTM in treating the clinical signs and symptoms of acute gout over an 8 day treatment period. Clinical effect was targeted to be greater than 50% pain reduction from baseline at approximately 72 hours after the first dose. Cohorts 1, 2 and 3 were administered dapansutrineTM doses of 500 mg BID, 500 mg QID and 300 mg dapansutrineTM (200 mg at 08.00 hour and 100 mg at 20.00 hour), respectively. Cohort 4 is currently enrolling subjects given 100 mg dapansutrineTM QD. VAS pain, general disability and walking disability scores were measured by daily diary and blood sampling was conducted on study days 0 (baseline), 3, 7 and 14 to assess PK and PD (including plasma cytokine levels, hsCRP, inflammasome activity, etc). Safety was measured over the duration of

the study with clinic visits on study days 0 (baseline), 3, 7, 14 and a follow-up telephonic visit on day 35.

Results A significant clinical and inflammatory cytokine response at Day 3 was seen in all dose groups and will be elaborated upon once the final datasets become available. There were no metabolic, physiological or hematological changes and all doses were well tolerated.

Conclusions On the basis of both the clinical response and the biomarkers, dapansutride™ is a safe and effective anti-inflammatory oral NLRP3 inhibitor in the treatment of acute gout with a broad therapeutic range and promise for further clinical development in this indication.

Acknowledgements We wish to acknowledge the gout patients willing to participate as well as Daniëlle Poeth and Dorine Baselmans for their commitment to the conduct of this study.

Potential investigators conflicts of interest DS is founder and CEO of Olatec, CS is CMO of Olatec, RB is COO of Olatec, CD is CSO and SAB member of Olatec, LJ is SAB member of Olatec.

Disclosure of Interest None declared.

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THE RELATION BETWEEN THE INFLAMMATORY STATUS OF HUMAN END STAGE OA SYNOVIUM AND LEVELS OF LOW DENSITY LIPOPROTEIN

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10.1136/annrheumdis-2018-EWRR2019.143

Career situation of first and presenting author Student for a master or a PhD.

Introduction High systemic levels of low density lipoprotein (LDL) is a shared metabolic risk factor for osteoarthritis (OA) and atherosclerosis, which may point to common biochemical pathways. One of the key events in atherosclerosis is formation and uptake of oxidized LDL (oxLDL) as a consequence of macrophage-mediated inflammation. In mice, high LDL levels enhanced synovial activation during development of collagenase-induced OA, and injection of oxidized LDL in naïve macrophage depleted joints led to infiltration of myeloid progenitor cells. However, a role for (ox)LDL in human OA has not yet been shown.

Objectives In the present study, we investigated whether the inflammatory status of OA synovium is associated to systemic and local levels of (ox)LDL.

Methods Blood was collected from patients planned to undergo total knee replacement and LDL serum levels were determined (n=16). After surgery, synovial explants from the same patients (2–8 per patient) were collected and incubated at 37 °C for 24 hours in presence of LDL (50 µg ApoB/mL), oxLDL (50 µg ApoB/mL) or control medium. Levels of IL-1β, TNF-α, IL-10 and MCP-1 secreted in the conditioned medium were measured using Luminex, and S100A8/A9 using ELISA. Immunohistochemical staining of ApoB was performed to assess presence and uptake of (ox)LDL in the synovium. Hematoxylin and eosin-stained sections were used to score cell infiltration and synovial hyperplasia.

Results Around 50% of the patients in our cohort showed synovial activation exemplified by high secretion levels of IL-1β, TNF-α and IL-10. Cellular infiltration and synovial hyperplasia was observed in the majority of synovial tissue samples, their grades did however not correlate with cytokine secretion.

Secretion levels of IL-1β positively correlated with serum levels of LDL and total cholesterol and did not correlate with BMI, suggesting a link that runs via systemic metabolic processes rather than increased weight-mediated joint loading. Staining of LDL associated protein ApoB in the synovia showed distinct speck-like staining within macrophages and endothelial cells especially, suggesting oxLDL accumulation in these cells. Unexpectedly, presence of high concentrations of LDL or oxLDL during incubation of synovial explants did not significantly affect secretion of IL-1β, TNF-α, IL-10 and MCP-1.

Conclusions IL-1β secretion by end stage OA synovium is associated to systemic levels of LDL. Intervention with high concentrations of LDL or oxLDL for 24 hours after synovium extraction did however not alter cytokine secretion, suggesting that LDL and synovitis are linked through more long-term local interactions, or alternatively via systemic inflammatory factors.

Disclosure of Interest None declared.

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EXTRACELLULAR VESICLES FROM PATIENTS WITH ANKYLOSING SPONDYLITIS CONVEY PROTEINS RELATED TO IMMUNE RESPONSE: NEW SIGNALING PATHWAYS?

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10.1136/annrheumdis-2018-EWRR2019.144

Career situation of first and presenting author Young investigator.

Introduction Ankylosing spondylitis (AS) is a chronic inflammatory disease, whose pathogenesis is still unclear. Many studies show the proteins in extracellular vesicle (EVs) would change regularly in many diseases. However, none of study has focused on the proteins profiles of serum-derived EVs in AS patients.

Objectives The study aim to detect the proteins profiles of serum-derived EVs in AS patients by Label-free-LC-MS/MS technology.

Methods EVs were isolated by ExoQuick-TC™ kit. The protein contents were evaluated by Label-free-LC-MS/MS technology. Venn diagram, volcano plot, hierarchical cluster, Gene Ontologies (GO), Encyclopedia of Genes and Genomes (KEGG) pathway and Protein-Protein Interaction (PPI) network analysis were constructed. Enzyme-linked immunosorbent assay (ELISA) was used to confirm the expression of the differently-expressed proteins.

Results Label-free-LC-MS/MS results indicated a total of 677 proteins were detected, of which 65 were unique to AS group, 545 were identified in both AS and control group, and 67 were unique to control group. A heat map of the hierarchical clustering revealed a clearly distinct expression of proteins in AS group, compared with control group. With the cut off criteria of $|\log_2(\text{fold-change})| \geq 1$, $p \leq 0.01$ and unique peptides ≥ 2 , 31 up-regulated proteins and 42 down-regulated proteins were found in the AS group. Among them, Serum amyloid A-1 (SAA1) protein and Complement C4-B were highly expressed in AS group. PPI network analysis yielded a highly clustered network (clustering coefficient: 0.584), with prothrombin (F2) and c-reactive protein(CRP) as the central/hub proteins. GO analysis of the biological process indicated that the differentially expressed proteins were significantly enriched