NANOCATALYTIC TREATMENT USING Ru@TiO₂ FOR RHEUMATOID ARTHRITIS VIA MACROPHAGE RE-POLARIZATION

Keywords: Animal models, Rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is an autoimmune disorder that results in synovitis and joint destruction[1]. Macrophages, one of the various inflammatory cells, particularly infiltrate the RA synovial tissue and cause sustained inflammation that promote RA. And the balance of pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages in RA joints is affected by overexpressed reactive oxygen species (ROS) and hypoxia synovium, which provides potential targets for the RA treatment.

Objectives: With the aim of alleviating the synovial inflammation in RA and restoring the balance of macrophage subtypes, we develop a catalytic nanoparticle named Ru@TiO₂, which can produce O₂ and scavenge ROS to reduce M1 macrophages, switch M1 phenotype to M2 phenotype.

Methods: Ru@TiO₂ was synthesized using the hydrothermal method. The morphology and nanoparticle size were observed using the transmission electron microscopy (TEM). The catalytic ROS-scavenging activities and O₂ generation ability were examined through O₂ scavenging, H₂O₂ catalytic elimination and O₂ generation assays. For in vitro experiment, the cytotoxicity of Ru@TiO₂ was conducted on RAW 264.7 cells was assessed by flow cytometry. To verify M1 to M2 macrophage phenotype transition and hypoxia alleviation, M1 and M2 markers and HIF-1α expression levels were evaluated by using qRT-PCR and Western Blot, respectively. In vivo, anti-inflammatory efficiency was observed after intra-articular injection of Ru@TiO₂ into collagen-induced arthritis mouse. The clinical scores and paw thickness for the inflamed joints with different treatments at various time points were recorded. Ultrasound (US) was used to assess the inflamed joints after treatment and RNA expression levels of M1 and M2 macrophage markers in synovial tissue were evaluated through qRT-PCR. The statistical significance among multiple groups was examined by using one-way ANOVA.

Results: The Ru@TiO₂ were fusiform in shape with an average size of 67 nm. The evaluation of catalytic ROS scavenging activities and O₂ generation performance of Ru@TiO₂ showed that Ru@TiO₂ can achieve about 60% scavenging ratio to O₂ and about 80% producing ratio to O₂ at 30min. Ru@TiO₂ also showed significantly increased H₂O₂ eliminating activity compared to the TiO₂. In vitro, low cytotoxicity of Ru@TiO₂ was displayed and the decrease of intracellular ROS level was most significant with Ru@TiO₂. In addition, M1 macrophage markers (TNF-α and IL-1β) and HIF-1α expression levels were most prominently declined with Ru@TiO₂. And the increase of M2 macrophage markers (Arg-1 and CD206) was most obvious when using Ru@TiO₂. In vivo, the arthritis scores, paw thickness and thickness of articular cavity assessed by US in Ru@TiO₂ group were the lowest when compared with other treated groups. Moreover, RNA expression levels of M1 and M2 macrophage markers in synovial tissue showed the similar tendency as in vitro.

Conclusion: In this study, the Ru@TiO₂ was successfully synthesized with efficient catalytic ROS scavenging activities and O₂ generation ability for the treatment of RA. In vitro, Ru@TiO₂ shows good cell biocompatibility and effective intracellular ROS scavenging ability. Moreover, Ru@TiO₂ alleviate the hypoxia condition in cells by producing O₂. Furthermore, the significant and efficient polarization of M1 to M2 macrophages and suppression of inflammation were achieved both in vitro and in vivo through the treatment of Ru@TiO₂ which indicated the therapeutic potential of Ru@TiO₂ for RA therapy.

References:

LIPOSOMAL ANTAGOMIR-155-5P RESTORES ANTI-INFLAMMATORY MACROPHAGES AND IMPROVES ARTHRITIS IN PRE-CLINICAL MODELS OF RHEUMATOID ARTHRITIS

Keywords: Cell biology, Innate immunity, Animal Models

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Background: RA is the most frequent systemic autoimmune disease that primarily affects multiple joints and causes irreversible bone and cartilage destruction in the absence of treatment [1]. Macrophages-macrophages are key players in the pathogenesis of RA and accumulate in the synovial tissue. Besides resident macrophages, blood monocytes can differentiate into monocyte-derived-macrophages that may join the synovium and have different phenotypes and
functions. The ‘classically activated macrophage phenotype’ is considered to be pro-inflammatory and contribute to RA pathogenesis by secreting pro-inflammatory cytokines. The ‘alternatively activated macrophage phenotype’ is considered to be regulatory and anti-inflammatory in tissues. Actually, there is a continuum from pro-inflammatory to anti-inflammatory macrophages, with high plasticity between the different states. We have previously shown that RA patients, and not patients with other inflammatory rheumatic diseases, have an impaired maturation of monocytes in anti-inflammatory-macrophages with increased differentiation in a pro-inflammatory-phenotype. We have found that an increased expression of miR-155 in monocytes/macrophages could be responsible for this defect and thus, could represent a new therapeutic target in RA [2].

Objectives: Our aim is to assess if the defect of monocytes polarization in anti-inflammatory-macrophages and the impact of miR-155 in this defect are present in 2 pre-clinical models of RA: the CIA (collagen-induce-arthritis) and STA mice (Serum transfert arthriti), both in which macrophages infiltration of synovium play a key role in pathophysiology. Then, we have tested a new therapeutic strategy to correct this defect using PEG-oligosomes containing antagonomiR-155-5p.

Methods: AntagomiR-155-5p or antagomiR-control were encapsulated in PEG-oligosomes of 100nm in size and -10mV in zeta potential with high antagoniR loading efficiency (above 80%). Mice were injected intravenously with 1,5nmol/100µL PEG-oligosomes containing antagoniR-155-5p or control after induction of arthritis.

Results: As in humans, we found that monocytes defect in anti-inflammatory-macrophages was associated with an increase of miR-155-5p in both mouse models. Moreover, we demonstrated the biodistribution of tagged-PEG-oligosomes to inflamed joints 1 hour after injection and as well as their subsequent liver’s accumulation after 48 hours, indicative of hepatic clearance, in arthritic mice. Subsequently, we demonstrated that treating mice with an antagoniR-155-5p encapsulated in PEG-oligosomes was able to decrease joint inflammation, to restore bone-marrow monocytes polarization in anti-inflammatory-macrophages, to reduce immune cells infiltration in synovial tissues, to increase the CD206+ and CD163+ tissue infiltrating macrophages and to decrease expression of mRNAs target of miR-155-5p, without any significant functional change in other immune cells including splenic B and T cells.

Conclusion: The injection of antagoniR-155-5p encapsulated in PEG-oligosomes allows delivering small RNA to monocytes/macrophages, lead to reduce joint inflammation in murine models of RA, providing a promising strategy in human disease.

REFERENCES:

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**OP0208** MECHANICAL UNLOADING PREVENTED ARTHRITIS IN THE RAT ADJUVANT-INDUCED ARTHRITIS MODEL

**Keywords:** Rheumatoid arthritis, Animal Models

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**Background:** Rheumatoid arthritis (RA) is the most common chronic inflammatory rheumatic disease, characterized by synovitis associated with progressive bone loss and joint swelling. The adjuvant-induced arthritis (AIA) model mimics the pathophysiological features of RA, with a very high prevalence and reproducibility. YAP plays a key role in synovial hyperplasia. It is a transcription factor which can be activated in response to inflammation but also by a mechanical stimulus as tissue stiffening. We already showed a decrease arthritis severity by inhibiting YAP in the AIA model [1]. Then, different mechanical challenges could potentially impact arthritis development by regulating YAP.

**Objectives:** The objective of this study was to investigate the impact of mechanical loading and unloading on the development of arthritis in the AIA model.

**Methods:** Arthritis was induced in female Lewis rats by injection of the adjuvant Mycobacterium butyricum, defining day (D)0. The AIA model normally develops arthritis at D10, with a peak of inflammation at D17 [2]. Rats were randomized into three groups: an AIA+mechanical loading group (n=11) with free access to an activity wheel from D0 to D17, an AIA+mechanical unloading group (n=11) by tail suspension from D0 to D17, and an AIA-only group (n=11) as positive control. Daily clinical monitoring (arthritic index and ankle circumference) was used to follow the progression and severity of arthritis. At D17, the ankles of the rats were collected to perform RT-qPCR.

**Results:** Arthritis onset was observed at the same time (D10) in the AIA control and AIA+mechanical loading groups with the same kinetic of arthritis index and ankle circumference. However, the majority of rats in the AIA+mechanical unloading group did not develop any signs of arthritis. At D17, gene expression of YAP and CRYF1 (YAP target gene), IL1B, IL6, RANKL, ACP5 (encoding TRACP), CTSK (encoding cathepsin K), MMP9, and MMP13 was decreased in the ankle of AIA+mechanical unloading rats compared to other groups. In the AIA+ mechanical loading group, rats stopped their physical activity at D10 which may explain the lack of clinical and molecular difference compared to the AIA+mechanical loading group at D17.

**Conclusion:** Mechanical unloading of the hindpaws by the suspension system strongly prevented arthritis by a reduced expression of pro-inflammatory genes, bone resorption, and bone degradation genes. The decrease in inflammation may be partly explained by the decrease in YAP transcriptional activity. Mechanical stress is therefore a key factor in inflammation during AIA. The precise mechanisms linking these two mechanisms are still under investigation.

**REFERENCES:**

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**OP0209** IMPACT OF CHRONIC INFLAMMATION AND COLLAGEN IV FRAGMENT CASSTATIN ON RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS AND ENDOTHELIAL CELL INTERACTIONS IN VITRO AND IN VIVO

**Keywords:** Cell biology, Synovium, Rheumatoid arthritis

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**Background:** Increased neovascularization in the inflamed synovium of patients with rheumatoid arthritis (RA) is a pathological feature in the pathogenesis of RA. Key players in RA progression are chronically activated RA synovial fibroblasts (RASF), which secrete proinflammatory factors (e.g. IL-6), proangiogenic...