ditions, respectively. CTLA-4 expression in MSC was analyzed on protein and on transcriptional levels by flow cytometry, immunoblotting, ELISA and by quantitative PCR, respectively. To assess the functionality of CTLA-4 expression, MSC were co-cultured with peripheral blood mononuclear cells (PBMC) isolated by density gradient centrifugation from venous blood of healthy donors. The cells were stimulated for 48h with PHA (5μg/ml) or left untreated and challenged using either CTLA-4-Ig or anti-CTLA-4-antibody, incubated under normoxic (∼18% O_2) or hypoxic (<1.5% O_2) conditions and analyzed for TNFα secretion by suspension assay.
Results: The MSC phenotype of bone-marrow derived cells could be verified according to their surface marker expression and their osteogenic and adipogenic differentiation capacity. On transcriptional level, MSC express both the full-length and - to a higher extent - the soluble CTLA-4 isoforms with a higher mRNA abundance under normoxic as compared to hypoxic conditions. Extra- and intracellular analysis of CTLA-4 expression on protein level, demonstrated a significant shift of the whole MSC population ($p<0{,}01$). CTLA-4 expression and secretion by MSC was confirmed using immunoblot and ELISA, respectively. Co-culture of MSC with PHA-activated PBMC significantly reduced the amount of secreted TNFα ($p<0{,}05$) which could be reversed by anti-CTLA-4-antibody ($p<0{,}05$), under both normoxic and compared to hypoxic conditions, respectively.
Conclusions: We clearly demonstrate the existence of CTLA-4 on hMSC and its functionality with regard to the inhibition of PHA-induced TNFα secretion by PBMC. We also demonstrate that the expression of CTLA-4 (i) contributes to the immunomodulatory capacity of hMSC and (ii) supports the '*immune privileged*' status of these cells.
Disclosure of Interest: None declared
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FRI0020 ALTERED BIOENERGETICS, MITOCHONDRIAL FUNCTION AND PRO-INFLAMMATORY PATHWAYS IN RA SYNOVIUM IN RESPONSE TO TOFACITINIB

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Background: Rheumatoid arthritis (RA) is a chronic joint disease, characterised by synovial inflammation and a shift in the metabolic profile of cells to a more destructive phenotype. The JAK-STAT signalling pathway is implicated in the pathogenesis of RA.
Objectives: To examine the effect of tofacitinib, a selective JAK inhibitor, on synovial cellular bioenergetics, mitochondrial function and subsequent pro-inflammatory mechanisms in RA.
Methods: *Ex-vivo* RA whole tissue synovial explants and primary RA synovial fibroblasts (RASFC) and were cultured with tofacitinib (1μM) for 24–72hrs. RASFC metabolism was assessed by the XF24-Flux-analyser and mitochondrial mutagenesis was quantified using a mitochondrial random mutation capture assay. Mitochondrial function was assessed for reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and mitochondrial mass (MM) using the specific cell fluorescent probes and differential gene expression by mitochondrial gene arrays or RT-PCR. Mitochondrial structural morphology was assessed by transmission electron microscopy. Lipid peroxidation (4HNE) was measured by specific ELISA. Dual staining of pSTAT3 and mitochondrial marker Cox-IV was demonstrated by confocal microscopy. The effect of tofacitinib (1μM) in RA synovial explant on markers of cellular bioenergetics and pro-inflammatory mediators, including cytokines and growth factors were quantified by ELISA, MSD multiplex assays and Real-time PCR.
Results: An initial screen demonstrated alterations in 18 key genes involved in mitochondrial function in RA synovial tissue in response to tofacitinib. Supporting this, tofacitinib inhibited ROS production, decreased the MMP and MM (all $p<0.05$), coupled with altered mitochondrial morphology. No effect observed for mtDNA mutations or 4HNE levels. Tofacitinib significantly inhibited the expression of glycolytic genes HIF1α, HK2, LDHA, GSK3A and PDK1 (all $p<0.05$) suggesting altered energy metabolism. This was paralleled by inhibition of baseline ECAR (glycolysis) with a concomitant increase in baseline OCR (oxidative phosphorylation), ATP production, maximal respiratory capacity and in the respiratory reserve in RASFC, confirming a bioenergetic switch in synovial cells in response to tofacitinib. Furthermore, we demonstrated co-localisation of pSTAT3 with Cox-IV in RASFC, suggested that in addition to nuclear transcription, pSTAT3 may also act as a mitoTF, regulating mitochondrial function directly. Finally, in RA whole tissue explants, tofacitinib significantly inhibited glycolytic genes HK2, GSK3A and PDK1 which was paralleled by a significant decrease in the spontaneous secretion of inflammatory mediators IL-6, IL-8, IL-1β, ICAM-1, VEGF, Tie2 and MMP1 (all $p<0.05$).
Conclusions: In this study, we describe a potential mechanism of action for tofacitinib, through reversing mitochondrial dysfunction and subsequent switch in cellular bioenergetics, in favour of a less glycolytic microenvironment leading to the reduction of inflammatory mediators. Thus, we have demonstrated that pathological cellular metabolism may be reversed by therapeutic treatment with tofacitinib.
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FRI0021 INTRAVENOUS INFUSION OF FUCOSYLATED BONE MARROW MESENCHYME CELLS IN PATIENTS WITH OSTEOPOROSIS. PRECLINICAL STUDY IN A MURINE MODEL

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Background: Osteoporosis is a skeletal disease characterized by systemic bone loss with an increased risk to bone fractures, associated with high morbidity and mortality[1]. Mesenchymal stem cells (MSC) from bone marrow (BM) are ideal candidates for the treatment of osteoporosis because they are able to differentiate to osteoblasts although its osteotropism is low[2]. Ex vivo fucosylation (adding a fucose residue) in α1–3 position of the CD44 antigen by use of the enzyme fucosyltransferase VI (FTVI) yields Hematopoietic cell E and L-selectin ligand (HCELL) in MSC increasing the affinity for E-selectin and osteotropism.
Objectives: To describe the safety and efficacy of human fucosylated MSC infused in an immunocompromised mice model (NOD/SCID).
Methods: 31 NOD/SCID mice were randomized to by tail vein injection: $1x10^6$ fucosylated MSC (n=13), $1x10^6$ MSC (n=14) or saline (n=4). Toxicities were evaluated by a clinical score, weight and histological assessment (heart, lung, liver, spleen, kidney, gonads, brain, bone and BM). RT-PCR array-based evaluation of the expression of human β-actin and β2-microglobulin genes was performed to study biodistribution of MSC. The maintenance of genetic integrity was evaluated during in vitro culture by karyotype. Additional samples of tibia and calota were to immunostained with a polyclonal Rabbit anti-human osteocalcina to demonstrate efficacy. Osteocalcin-positive cells were identified by a dark-brown cytoplasmic precipitate.
Results: There was no unexpected death and none of the mices had any acute toxicity. The histology of heart, liver, kidney, spleen, gonads, brain, bone and bone marrow was normal in all mice. There were localized areas of lung inflammation in 15%, 42% and 25% of mice infused with fucosylated MSC, MSC, and saline, respectively without significance differences (p=0.28). Biodistribution was normal with except one mice (infused with MSC non-fucosylated) showed an expression of human RNA β-actin and β2-microglobulin in the lung sample taken after 12 weeks of intravenous infusion. Osteoblasts were seen in 100% of mices infused with fucosylated MSC, in 62.5%% of animals infused with MSC alone, and none in saline group (p=0.01). NOD/SCID mice infused with fucosylated MSC presented a higher number of osteoblasts positive for osteocalcin in 10 high-power fields (400x) of tibia and calvarium sections that non-fucosylated (32 vs 5.5) (p=0.0082). Human osteoblasts were detected inside the bone from the fifth to the twelfth week after infusion.
Conclusions: Intravenous infusion of human fucosylated MSC is safe and effective in guiding the cells to bone with a higher potential to ossification in NOD/SCID mices that non-fucosylated MSC. These results are allowing to start a human clinical trial with intravenous fucosylated MSC as treatment in patients with osteoporosis.
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Rheumatoid arthritis - etiology, pathogenesis and animal models

FRI0022 GLUTAMINE METABOLISM PLAYS A KEY ROLE IN THE CELL GROWTH OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Background: Many signaling pathways activated under inflammatory and hypoxic conditions have profound effects on intracellular metabolism to support cell growth and survival. The recent findings of cancer-specific metabolic changes, including increased glucose and glutamine consumption, has provided new therapeutic targets for consideration. The microenvironment in inflamed joints in RA is also characterized by hypoxia and low concentration of nutrients, and fibroblast-like synoviocytes from RA patients (RA-FLS) is known to have several tumor-like characteristics. However, the role of glucose and glutamine metabolism in the aberrant proliferation of these cells is unclear.