Student Travel Award Recipient

The Therapeutic Potential of Bovine Milk-Derived Extracellular Vesicles for Treatment of Osteoarthrits Patients

Bartijn Pieters, Radboud University Medical Center, Nijmegen, The Netherlands

Bartijn Pieters[1], Onno Arntz[1], Danny Kartoidjojo[1], Anouk Feitsma[2], Joost van Neerven[2], Peter van der Kraan[1], Fons van de Loo[1]

1. Experimental Rheumatology, Radboud University Medical Center, Nijmegen, The Netherlands

2. FrieslandCampina, Amersfoort, The Netherlands

Introduction: Bovine milk is a rich source of extracellular vesicles, which are small phospholipid bilayer bound structures that facilitate intercellular communication. It has been shown that these vesicles are able to survive the harsh conditions of the intestinal track and are believed to be taken up into the bloodstream by consumers. We, and others, have highlighted the anti-inflammatory potential of EVs isolated from bovine milk in animal models of experimental arthritis [1,2]. However, little is known how this translates to the human situation. In this study, we investigate the effects of bovine milk-derived EVs (MEVs) on cells of the cartilage (articular chondrocytes) and from the joint capsule (synovial fibroblasts) derived-from osteoarthritis (OA) patients. OA has long been considered a disease of the cartilage due to mechanical stress evoked either by trauma or overloading of the joint especially in combination with loss of chondrocyte function due to aging. Evidence now emerges that in a high percentage of OA patients signs of synovial inflammation can be detected at the early stage of disease. Currently there is no cure and pain killers are the only available drugs for these patients. We investigate whether bovine milk-derived EVs have the potency to reduce joint pathology.

Methods: MEVs were isolated from commercial skimmed cow milk using a standard differential ultracentrifugation protocol. Particle concentration, size and floating density were assessed by NTA analysis and sucrose density gradient, respectively. Articular chondrocytes and primary fibroblast-like synoviocytes (FLS) from OA patients were stimulated for 24hrs and 48hrs with MEVs and gene expression profiles were studied by RT-qPCR. Additionally, short stimulations (2hrs) were performed, in the presence of an anti-TGFβ1,2,3 antibody, to study direct TGFβ-receptor activation.

Results: Stimulation of articular chondrocytes with 10-100µg/ml MEVs was able to effectively reduce expression of cartilage destructive enzymes (ADAMTS5, MMP1, MMP3) and inflammatory mediators (IL6, IL8, TNFα) that play key roles in the progression of OA. Additionally, we observed a significant increase in expression of TIMP3, a potent inhibitor of above mentioned cartilage destructive enzymes. Stimulation of primary FLS showed similar results, with marked reduction of catabolic enzymes (ADAMTS5, MMP1) and also increased in TIMP3 levels. The reduction in inflammatory mediators was however not found, and in contrast IL6 was significantly

increased in FLS after exposure to MEVs. Short exposure of chondrocytes to MEVs led to induction of early TGFβ response genes (JUNB, SMAD7, PAI), which was completely blocked using an anti-TGFβ1,2,3 antibody.

Conclusion: Human articular chondrocytes and synovial fibroblasts exposed to MEVs show reduced destructive and inflammatory potential. The induction of early TGFβ response genes after short incubations confirms the presence of active TGFβ, which could explain, in part, the anti-inflammatory and reduced catabolic profiles found. These findings highlight the therapeutic potential of MEVs in osteoarthritis, where inflammatory and catabolic mediators are responsible for joint pathology and subsequent loss of mobility. However, more in vitro work is required to compare different milk sources (e.g. raw milk, colostrum, whey) to find the most potent MEV, and to perform preclinical animal studies before this therapy can be tested in patients.