DYNAMICS OF EXTRACELLULAR VESICLES CARGO FROM EQUINE SYNOVIAL FLUID DURING OSTEOARTHRITIS PROGRESSION

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Introduction

Osteoarthritis (OA) is a progressive degenerative joint disease characterized by inflammation, extracellular matrix degradation, and altered intercellular communication. Extracellular vesicles (EVs) released into the synovial fluid play critical roles in mediating cellular responses and may serve as potential biomarkers reflecting disease progression. This study aimed to to evaluate the expression of inflammatory and cartilage matrix degradation biomarkers in EVs isolated from the synovial fluid of horses with experimental OA.

Material & Methods

Ethics Committee (CEUA-FMVZ)- Protocol: n° 000.204
Synovial fluid samples were collected from horses at day −7 (baseline, before induction), day 0, 7, 14, and 35 after OA induction with monoiodoacetate (MIA). EVs were isolated by differential ultracentrifugation and characterized by nanoparticle tracking analysis (NTA) and flow cytometry. Total RNA was extracted from EVs, and the expression of IL-6 and MMP-13 genes, associated with inflammation and cartilage matrix degradation, respectively, was quantified by RT-qPCR. The 2-ΔΔCt comparative critical threshold method was used to calculate the relative quantity, with results expressed as the mean ± standard deviation.

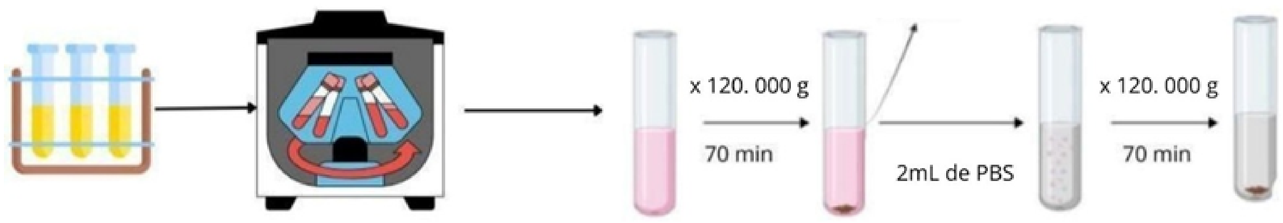


Fig 1: Two sequential differential ultracentrifugation (120.000G/ 70 min, 40.100 rpm, k-factor 36).

Results

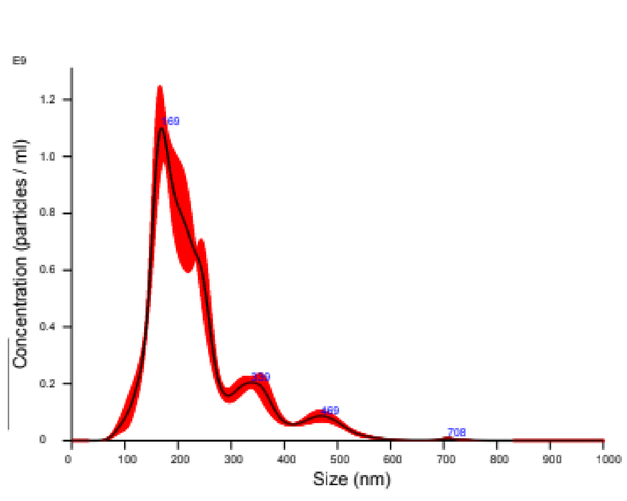


Fig 2: a.Size and concentration by NTA: 180.0 +/- 2.7 nm; 2.33x1010 +/- 4.21x108 particles/ mL. b.Positive fluorescence for CD9, CD63, CD81, ALIX, and negative for Calnexin in relation to the events obtained in flow cytometry.

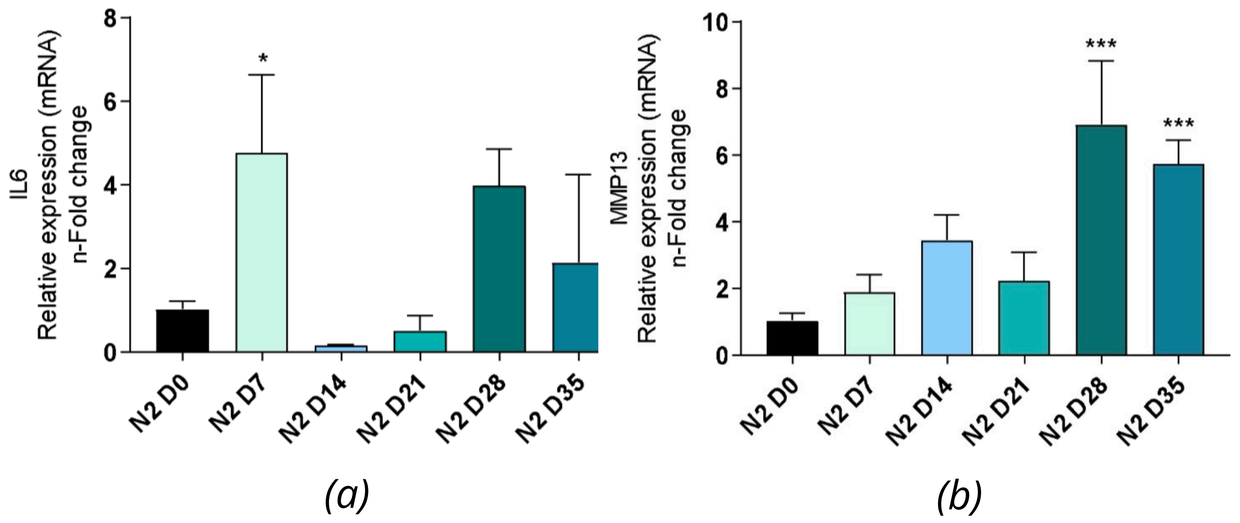


Fig 3: a. Significant increase in IL-6 gene expression at D7 and D14; 6.b. MMP-13 increased gene expression in EVs isolated from the synovial fluid at D7 e D14.

Discussion and Conclusion

Gene expression analysis revealed a significant increase ($p<0.05$) in IL-6 at day 7, corresponding to the early inflammatory phase induced by MIA. In the chronic stages (days 28 and 35), MMP13 expression was markedly elevated, reflecting cartilage degradation and type II collagen network breakdown. The temporal pattern suggests that IL-6 acts as an early mediator of inflammation, promoting MMP release and explaining the correlated peaks and declines observed between these biomarkers during disease progression.

It suggested that the temporal variation of inflammatory and catabolic biomarkers captured by EVs during OA progression reinforces the potential of synovial EVs as a source of more specific and sensitive molecular biomarkers for early detection and monitoring of OA in horses. Finally, focusing the analysis on EVs reduces interference from abundant synovial proteins and enzymes, increasing the accuracy of molecular target detection. Thus, it is suitable to direct studies towards the development of a product to anticipate diagnosis.

Acknowledgments

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