Abstract N°: 2027

APPA (Apocynin and Paeonol) reduces ROS production and Senescence in human articular chondrocytes

Mercedes Fernandez-Moreno*[1], Nicholas Larkins[2], Alan Reynolds[2], Tamara Hermida Gómez[1, 3], Francisco J. Blanco[1]

[1]Instituto de Investigación Biomédica de A Coruña (INIBIC). Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas. Universidade da Coruña (UDC). CICA-INIBIC. , Servicio de Reumatología., A Coruña, Spain, *[2]AKL Research & Development, Stevenage Bioscience, Stevenage SG1 2FX., United Kingdom*, *[3]Centro de investigación biomédica en Red, Bioingenieria, Biomatereial y Nanomedicina, (CIBER-BBN)., Madrid, Spain*

Background:

Disease modification is not yet possible for osteoarthritis (OA). Mitochondrial ROS and pro-inflammatory cytokines are involved in the pathogenesis of OA and are potential therapeutic targets. APPA, a combination of apocynin (AP) and paeonol (PA), has the potential capacity to modulate synthesis of pro-inflammatory stimuli.

Objectives:

To investigate the anti-inflammatory effect of APPA in human articular chondrocytes and cartilage.

Methods:

Tissue and chondrocytes from human OA cartilage were isolated. The effect of APPA on chondrocyte viability was analyzed using MTT. IL-1β 10 ng/mL and LPS 10 ng/mL were used as pro-inflammatory stimuli. ROS production was evaluated by flow cytometry using DCFH-DA and MitoSoxRed. The percentage of senescent cells was evaluated through the quantification of Fluorescein di-β-D-galactopyranoside (FDG) by flow cytometry. The effect of APPA on gene expression of pro-inflammatory cytokines (IL-8 and TNF-α) and enzymes degrading cartilage (MMP-13 and MMP-3) were analyzed in chondrocyte and cartilage by RT-PCR. Quantification of Toluidine Blue (TB) staining in cartilage was performed to evaluate proteoglycans content using software ImageJ/Fiji. Release of Glycosaminoglycan (GAGs) into the supernatant was quantified using BlyscanTM Glycosaminoglycan assay. Statistical analyses were performed with GraphPad Prism v6.

Results:

Chondrocytes, incubated in presence of APPA 10 µg/mL for 24 h had viability >85%, reduced cytoplasmic ROS (p=0.028) and mitochondrial anion superoxide production induced by LPS 10 ng/mL (p=0.057). Chondrocytes incubated in presence of APPA 10 µg/mL for 2 hours contained significantly fewer senescent cells (p=0.0079). APPA significantly reduced the gene expression induced by IL-1*β* 10 ng/mL in chondrocytes of *IL-8*, *TNF-α*, *MMP-13* and *MMP-3*. *Cartilage* incubated with APPA 60 and 100 µg/mL for 48 h showed decreased the *MMP-3* gene expression induced by IL-1β (p=0.021 and p<0.0001 respectively). Quantification of TB showed that APPA 60 and 100 µg/mL during 48h increased the proteoglycans in intermedial layer, which had been decreased through the incubation with IL-1β (p=0.0018 and p=0.018 respectively). Quantification of release GAGs into the supernatant decreased significantly when the cartilage explants were incubated for 48h in presence of APPA 100 µg/mL (p=0.028).

Conclusion:

APPA has a clear anti-inflammatory effect on human articular chondrocytes, and could reduce extracellular matrix degradation of cartilage. This could be mediated by the capacity to modulate ROS production and reduce senescence

Acknowledgements:

Disclosure of interest: Mercedes Fernandez-Moreno: None declared, Nicholas Larkins Shareholder of: I am a shareholder in AKL Research and Development Ltd, Alan Reynolds Shareholder of: I have share options in AKL Research and Development Ltd

, Speakers bureau: I have not been a paid speaker for a pharma company - at least not since 2008 whichI think is outside the scope of this

, Consultant of: The last time I was a paid consultant was in 2017 when I acted as a consultant for Avillion and Norgine, Employee of: I am also an employee of AKL Research and Development Ltd, Tamara Hermida Gómez: None declared, Francisco J. Blanco Speakers bureau: Lilly

Pfizer

Sanofi

Galapagos, Consultant of: Lilly

Pfizer

Sanofi

Galapagos, Grant/research support from: Lilly

MSD

Merck Serono

Pfizer

Pierre-Fabra

Roche

Sanofi

Servier

UCB

Abbvie

Amgen

Bioiberica

Bristol Mayer

Celgene

Celltrion

Cellerix

Grunenthal

Gebro Pharma

AKL Research and Development Ltd