3. Bone/cartilage biology

A39 INFLAMMATORY RESPONSE IS MODULATED BY MITOCHONDRIAL DYSFUNCTION IN CULTURED NORMAL HUMAN CHONDROCYTES

C Vaamonde-García, N Valcarcel-Ares, R Riveiro-Naveira, B Lema, F J Blanco, M J López-Armada *INIBIC, Spain*

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Purpose The molecular inflammation hypothesis of ageing implies that the molecular activation of proinflammatory genes by altered redox-sensitive cellular signal pathway might serve as a bridge between normal ageing and age-related diseases such as osteoarthritis (OA). We studied the effect that dysfunction of the mitochondrial respiratory chain (MRC), the main cellular source of reactive oxygen species (ROS), could induce on the inflammatory response in cultured normal human chondrocytes, specifically in interleukin 8 (IL8) and cyclooxygenase 2 (COX-2) expression.

Method Antimycin A (AA) (10 µg/ml) and oligomycin (Oli) (10 µg/ml) were employed as inhibitors of complex III and V of MRC, respectively. IL1β or tumour necrosis factor α (TNFα) were used as inducers of the inflammatory response. Protein and mRNA IL8 and COX-2 expression were analysed by cytometry and real-time PCR and prostaglandin E2 (PGE2) levels were assayed by ELISA. To identify underlying mechanisms responsible for the inflammatory response, a chemical and a natural ROS scavenger, N-acetyl-L-cysteine (NAC) (40 mM) and resveratrol (RESV) (250 µM) respectively, were employed. Finally, the effect of nuclear factor κB (NF-κB) was studied by the inhibitor BAY-117085 (5 µM).

Results We first tested if mitochondrial dysfunction induced by AA or Oli could modulate the response induced by IL1 (5, 1.5 and 0.5 ng/ml) on IL8 expression. The results showed that the pretreatment of chondrocytes with AA or Oli for 1 h increased significantly the expression of IL8 induced by IL1, both mRNA and protein level (214.06±35.22% AA+IL1 or 292.06±49.02% Oli+IL1 vs 100% IL1 1.5 ng/ml, $p<0.01$). Similar effects were observed with 10 ng/ml TNF. In addition, the intensification of the inflammatory effects of cytokines by mitochondrial dysfunction was counteracted by the addition of a chemical and a natural ROS scavenger (10.75±2.97% NAC+AA+IL1 or 18.10±5.04% RESV+AA+IL1 vs 100% AA+IL1, $p<0.01$). When the role of NF-κB was

investigated, the results showed that preincubation of cells for 1 h with BAY-117085 significantly reduced to $26.53 \pm 16.80\%$ the production of IL8 protein induced by AA+IL1 (100%). Finally, we examined whether mitochondrial dysfunction could modulate COX-2 expression induced by IL1; both COX-2 mRNA and protein levels ($399.96 \pm 79.93\%$ Oli+IL1 vs 100% IL1 and $90.66 \pm 7.97\%$ Oli, $p < 0.001$) and its product PGE2 ($13\,522 \pm 6845\%$ Oli+IL-1 vs $1844 \pm 1419\%$ IL1 and 6825 ± 3813 pg/250 000 cells Oli, $p < 0.01$) increased significantly in Oli-pretreated chondrocytes induced by IL1.

Conclusion These findings suggest that mitochondrial dysfunction may be one of the factors that sensitise chondrocytes to increase the cytokine-mediated inflammatory response in OA and ageing.