Regulation by reactive oxygen species of interleukin-1β, nitric oxide and prostaglandin E₂ production by human chondrocytes

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Introduction
Cartilage degradation is a major process in the pathogenesis of arthritis. This process is tightly regulated at local level by networks of hormones, cytokines and reactive oxygen species (ROS). The oxidative power of the ROS conducted to tissue damages but also to the activation of some nuclear transcription factors such as NF-κb and AP-1. These factors regulate the expression of a number of genes involved in inflammation and especially in the pathogenesis of rheumatoid arthritis and osteoarthritis.

Aims of the study
This study was designed to investigate the action of the antioxidants N-monomethyl-L-arginine (L-NMMA) and N-acetylcycteinyl (NAC) on chondrocyte IL-1β, the nitric oxide (NO) and prostaglandin E₂ (PGE₂) productions.

Materials and Methods
Human chondrocytes were enzymatically isolated from osteoarthritic knee cartilage and then maintained in culture in suspension for 48h in the absence or presence of lipopolysaccharide (LPS) (10 µg/ml), L-NMMA (0.05 to 5 mM) or NAC (0.1 to 2 mM). IL-1β-stimulated IL-6 in both N and OA chondrocyte cultures (fig. 1). At 30 µM, it reduced NO₂/NO₃ (white columns) and PGE₂ (hatched columns) productions by chondrocytes. Human chondrocytes were cultured for 48h in the absence or in the presence of LPS (10 µg/ml). NO and PGE₂ productions were directly quantified in the culture conditioned medium by specific immunoassays. Nitrite was measured in the culture supernatants by a spectrophotometric method based upon the Griess reaction. Cyclooxygenase-2 (COX-2), inducible NO synthase (iNOS) and IL-1β gene expressions were quantified by reverse transcription of mRNA followed by real time and quantitative polymerase chain reaction (LightCycler SYBR Green I technology, Roche diagnostics, Brussels, Belgium). COX-2 protein expression was analysed by Western-blot.

Results
1) Production of NO and PGE₂ by LPS-treated chondrocytes

LPS increased NO and PGE₂ productions by human chondrocytes whereas IL-1β remained undetectable (fig.2).

Furthermore, the addition of L-NMMA resulted in a significant enhancement of LPS-stimulated PGE₂ production suggesting a negative effect of NO on PGE₂ synthesis (fig. 3A). Additionally NAC increased both the basal and LPS-stimulated NO production without significant effect on PGE₂ synthesis (fig. 3B).

2) Effects of L-NMMA, NAC on NO and PGE₂ productions by human chondrocytes

As expected, L-NMMA, a competitive inhibitor of the NO synthase activity, dose dependently decreased both basal and LPS-stimulated NO production (fig. 2). The maximal effect was achieved for 0.5 mM of L-NMMA.

3) Effects of L-NMMA and NAC on IL-1β, iNOS and COX-2 mRNA expression

After 48h of incubation, LPS induced a marked increase of the IL-1β, iNOS and COX-2 mRNA levels (fig. 4). Gene expressions were not significantly modified by L-NMMA (fig. 4). NAC enhanced IL-1β and iNOS mRNA levels (fig. 4A) and without affecting the COX-2 gene expression (fig. 4B).

Discussion

1) The inhibition of NO production by L-NMMA is accompanied by an increase of PGE₂ production suggesting a negative feedback of NO on PGE₂ synthesis.

2) L-NMMA does not modify both the COX-2 mRNA level and protein synthesis. These observations suggest that the overproduction of PGE₂ resulting from the L-NMMA treatment is directly related to the control of the enzymatic activity of cyclooxygenase.

3) NAC increases NO synthesis but is without effect on PGE₂ production.

4) NAC upregulates IL-1β and iNOS gene expression.

In conclusion, these findings suggest that antioxidant therapy could have contradictory effects as a function of the molecule administered especially the oxygen radical targeted. To block NO could result in an increase of PGE₂ production, which is, clearly identify as an inflammatory and mediator inflammatory actions.