Regulation by reactive oxygen species of interleukin-1ß, nitric oxide and prostaglandin E_2 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-kb 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-acetylcysteine (NAC) on chondrocyte IL-1ß, the nitric oxide (NO) and prostaglandin E_2 (PGE_2) 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 1 mM) or NAC (0.1 to 2 mM).
IL-1ß and PGE_2 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-1ß 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 PGE_2 productions by human chondrocytes whereas IL-1ß remained undectable (fig.1).

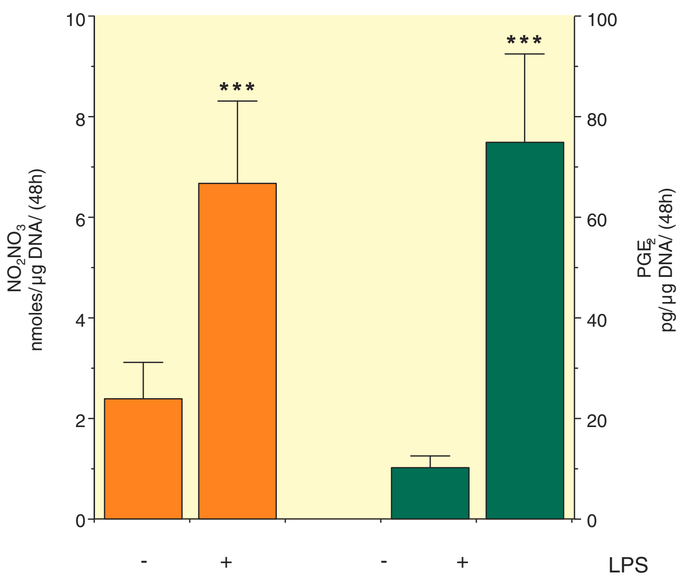

Figure 1.
NO_2/NO_3 (white columns) and PGE_2 (hatched columns) productions by human chondrocytes cultured for 48 h in the absence or in the presence of LPS (10 µg/ml). Results are the means ± SEM of eight different cultures performed with cartilage samples coming from eight different donors. Each culture condition was realised in triplicate. Comparison of mean values was performed by Student-t test. LPS treated groups are significantly different from the untreated groups with a $p < 0.001$***.

In the basal conditions, ACE, at the concentration of 30 µM, significantly decreased IL-6 production in both N and OA chondrocyte cultures. No significant effect was observed at the lowest concentrations (data not shown).
In the presence of IL-1ß, ACE at the concentrations of 1 and 6 µM significantly inhibited IL-6 production in OA but not in N chondrocyte cultures (fig. 2). At 30 µM, it reduced
IL-1ß-stimulated IL-6 in both N and OA chondrocyte cultures.
IL-8 production was not significantly modified by ACE whatever the concentration tested and the origin of the cartilage.

2) Effects of L-NMMA, NAC on NO and PGE_2 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.x
NO production was dose-dependently enhanced by NAC with a maximal effect at 1 mM.

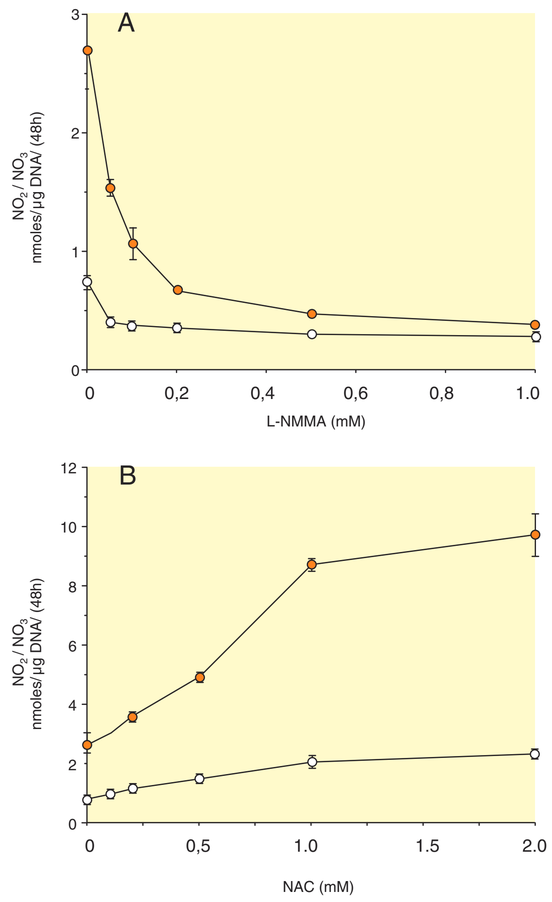

Figure2.
Dose effect of L-NMMA (A) and NAC (B) on NO production. Human chondrocytes were cultivated for 48h in the absence (-o-) or in the presence (--) of LPS (10 mg/ml. Results are mean ± SD of triplicate.

Furthermore, the addition of L-NMMA resulted in a significant enhancement of LPS-stimulated PGE_2 production suggesting a negative effect of NO on PGE_2 synthesis (fig. 3A). Inversely, NAC increased both the basal and LPS-stimulated NO production without significant effect on PGE_2 synthesis (Fig. 3B)

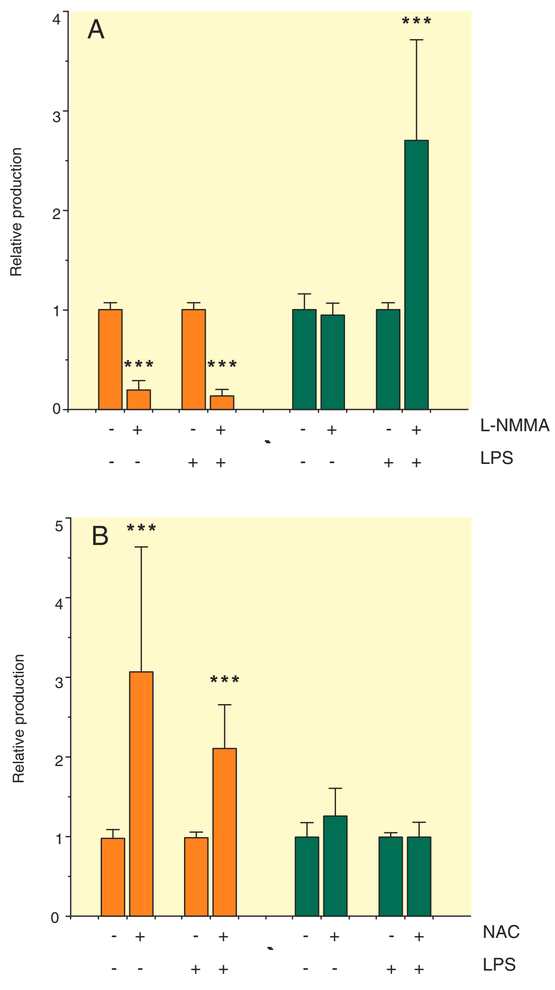

Figure 3.
Effects of L-NMMA and NAC on the relative productions of NO_2/NO_3 and PGE_2 by chondrocytes. Human chondrocytes were cultured for 48h in the absence or in presence of LPS (10 µg/ml) and with or without with L-NMMA (0.5 mM) (A) or NAC (1 mM) (B). NO_2/NO_3 (white columns) and PGE_2 (hatched columns) concentrations were measured in conditioned culture supernatants and were normalised to the production obtained in the absence of the drug. Each condition was tested in triplicate and the experiment was repeated with chondrocytes isolated from 7 donors. Results are expressed as mean ± SD. Comparison of mean values was performed by Student t-test. Drug treated groups are significantly different from the untreated groups: ***$p < 0.001$.

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

After 48h of incubation, LPS induced a marked increase of the IL-1ß, iNOS and COX-2 mRNA levels (fig. 4).

Gene expressions were not significantly modified by L-NMMA (fig. 4). NAC enhanced IL-1ß and iNOS mRNA levels (fig. 4A and B) without affecting the COX-2 gene expression (Fig. 4C).

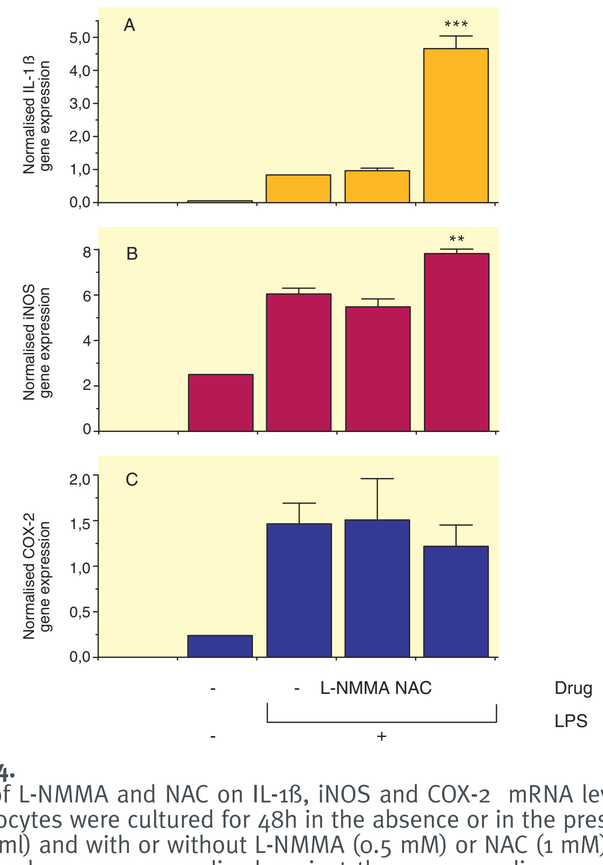

Figure 4.
Effect of L-NMMA and NAC on IL-1ß, iNOS and COX-2 mRNA levels. Human chondrocytes were cultured for 48h in the absence or in the presence of LPS (10 µg/ml) and with or without L-NMMA (0.5 mM) or NAC (1 mM). The mRNA copy numbers were normalised against the corresponding copy number of GAPDH mRNA. Data represent mean ± SD of triplicate. Comparison of mean values was performed by Student t-test (drug treated groups are significantly different from the untreated groups: *** $p < 0.001$, ** $p < 0.01$).

4) Effects of L-NMMA and NAC on COX-2 protein

COX-2 expression was induced by LPS (Fig. 5). L-NMMA and NAC had no significant effect on COX-2 expression.

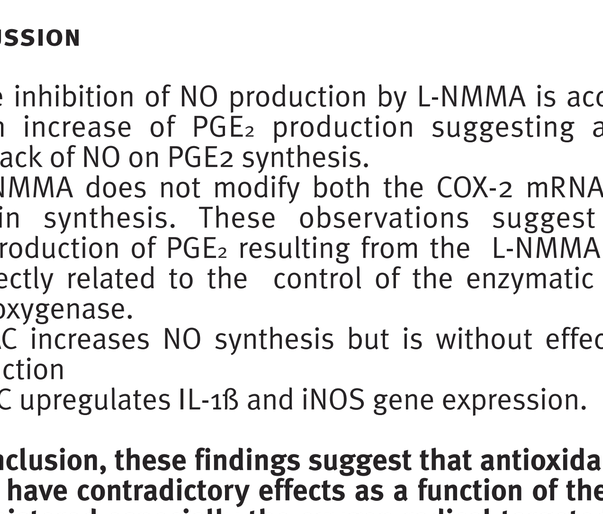

Figure 5.
Western-blot analysis of COX-2 in human chondrocyte extracts. Cells were cultured for 48h in the absence or in the presence of LPS (10 mg/ml).
L-NMMA (0.5 mM) or NAC (1 mM) were added at the beginning of culture. The cells were lysed and protein extracts were analysed by Western-blot with a specific antiserum for COX-2. Ovine COX-2 (400 ng) was used as standard.

Discussion

1) The inhibition of NO production by L-NMMA is accompanied by an increase of PGE_2 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 PGE_2 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 PGE_2 production
4) NAC upregulates IL-1ß 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 PGE_2 production, which is, clearly identify as an inflammatory and mediator.nflammatory actions.