Regulation by reactive oxygen species of pro-inflammatory genes in chondrocytes

Mathy-Hartert M (1), Martin G (2), Boumediene K (2), Pujol J-P (2), Reginster J-Y(1), Henrotin Y (1).
(1) Bone and Cartilage Metabolism Research, University of Liege, Belgium;
(2) Laboratory of Connective Tissue Biochemistry, University of Caen, France.

Introduction

Reactive oxygen species (ROS) were identified as potent mediators in cartilage tissue destruction and synovial membrane inflammation. These ROS, mainly produced by polymorphonuclear leukocytes and macrophages, were essentially superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO). NO and $O_2^{\cdot-}$ react together to form peroxynitrite ($ONOO^-$) a powerful oxidant. Chondrocytes exposed to soluble factor such as interleukin-1ß (IL-1ß) or lipopolysaccharide (LPS) are also capable to produce ROS. This research work was designed to investigate the effect of ROS on the expression by chondrocytes of pro-inflammatory genes such as interleukin (IL)-1ß, -6, -8, cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS).

Materials and Methods

Human chondrocytes culture and oxidative stress

Human chondrocytes were enzymatically isolated from osteoarthritic knee cartilage and then submitted to exogenous ROS produced by a 3 hours incubation in HBSS with
- S-nitroso-N-acetyl-D, L-penicillamine (SNAP) (10^{-4}M) a NO donor,
- 3-Morpholinosydnonimine (SIN-1) (10^{-4}M) a $ONOO^-$ generating molecule,
- $ONOO^-$ chemically synthesised (10^{-5}M)
- or H_2O_2 (10^{-4}M).

After washings, the cells were cultured for the next 24 hours with or without lipopolysaccharide (LPS) (10 µg/ml) or IL-1ß (10^{-11}M).

RNA was isolated by SV total RNA Isolation System (Promega) and was converted in cDNA by reverse trancriptase treatment. Amplification was performed with a spectrofluorometric thermal cycler (LightCycler, Roche Diagnostics, Brussels, Belgium). PCR reaction was performed by using the LightCycler-FastStart DNA master Sybr Green I (Roche Diagnostics, Brussels, Belgium).

Electrophoretic mobility shift assay

Nuclear extracts were prepared from 20 10^6 cells. Protein concentration was estimated by Biod-Rad Protein Assay (Bio-Rad, Nazareth, Belgium). Gel retardation assay were performed with oligonucleotides NF-kappa B consensus : AGTTGAGGGGACTTTCCCAGGC.
Nuclear extracts (10 µg) were incubated for 15 min at room temperature with the probe (20 fmol) and samples were fractionated by electrophoresis on a 5 % polyacrylamide gel

Results

Effects of exogenous ROS exposure on iNOS, IL-6, IL-8, IL-1ß, COX-2 gene expression.

Under basal conditions, low expression of the inflammatory genes was obtained. The drugs did not affect these basal expressions.
The inflammatory genes were highly expressed in response to LPS and IL-1ß.

SNAP significantly down-regulated LPS-induced IL-1ß, IL-6, lL-8 and COX-2 gene expression, whereas SIN-1 and $ONOO^-$ were inefficient. SNAP fully blocked iNOS gene expression while SIN-1 and $ONOO^-$ partially decreased LPS-induced iNOS mRNA.
When chondrocytes were activated by IL-1ß, SIN-1, ONOO- and H_2O_2 drastically decreased gene expressions while SNAP has no effect.

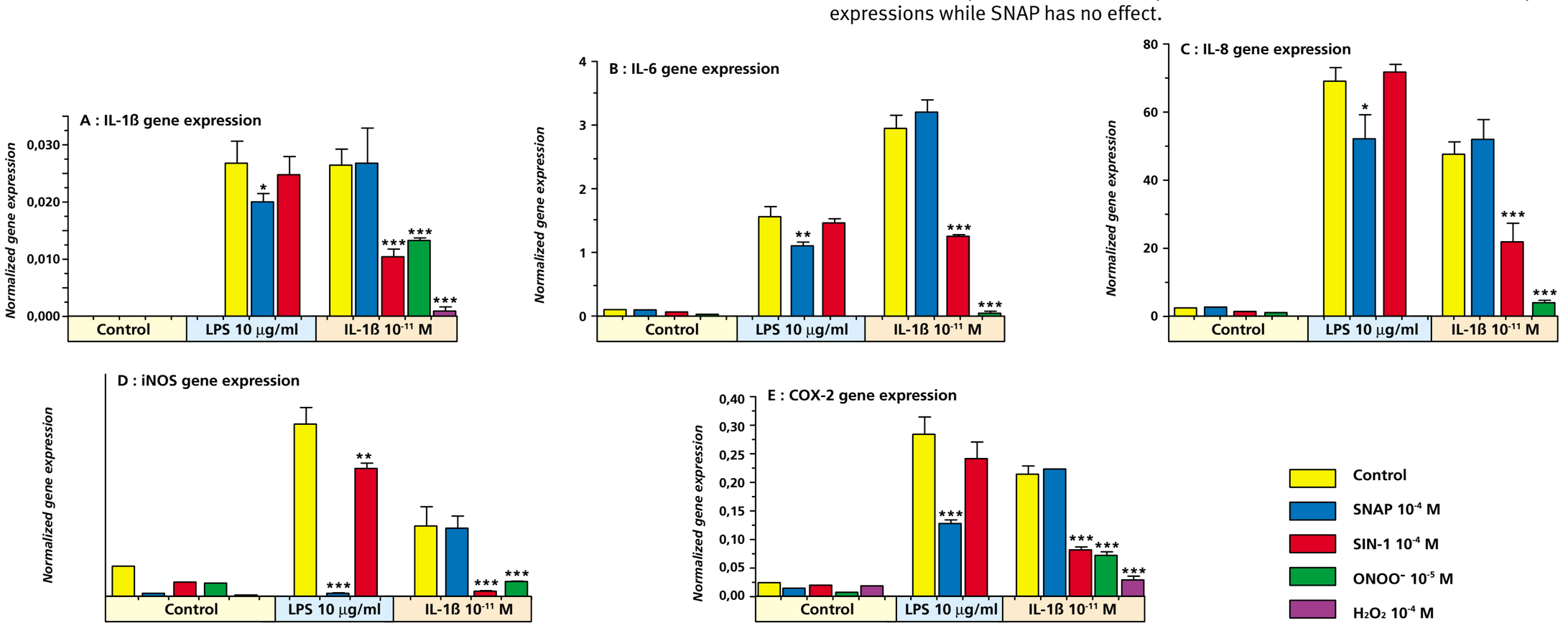

Figure 1 : Effect of oxidative stress on activation of chondrocyte gene expression

Effects of exogenous ROS on NF-kB DNA binding activity in chondrocytes.

Il-1ß-induced gene expression was accompanied by NF-kB DNA binding activation.
After 3 hours treatment, H_2O_2 and $ONOO^-$ but not SNAP activated NF-kB DNA binding, and potentiated IL-1ß induced NF-kB DNA binding

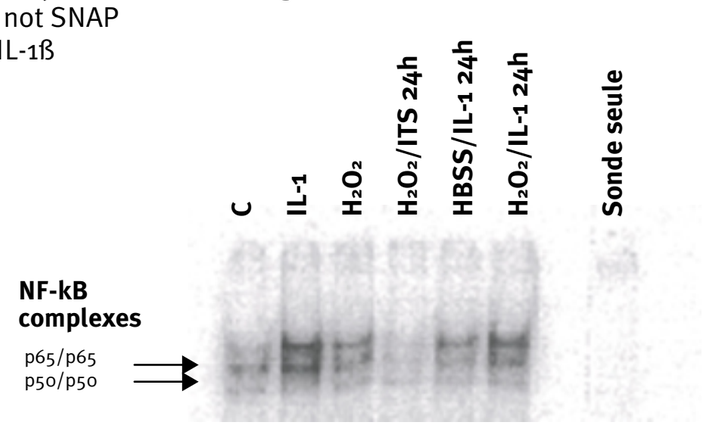

Figure 2 : Effect of ROS on NFkB DNA-binding activity in chondrocytes.
The cells were treated with or without IL-1ß (10^{-11}M) or H_2O_2 (10^{-4}M) for 3 hours in HBSS before preparing nuclaer extracts for analysis by EMSA with NFkB (well 1,2, and 3).
After H_2O_2 treatment, cells were washed and were untreated or treated with IL-1ß (10^{-11}M) for 24 h. Nuclear extacts were analysed for NFkB binding activity (well 4,5 and 6).

Conclusion

LPS and IL-1ß-induced gene expressions are differently regulated by ROS. The LPS signalling pathway was down regulated by NO whereas IL-1ß signalling pathway was sensitive to $ONOO^-$. This work was supported by an unrestricted grant of UCB Pharma.