Production of inflammatory mediators by human chondrocytes : Effects of aceclofenac and its metabolites.

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Introduction

Aceclofenac (ACE) is a non steroidal anti-inflammatory drug, which has been shown to exhibit a good clinical efficiency in joint diseases. In healthy human, ACE is rapidly metabolised in human hepatocytes and human microsome to form 4'-hydroxyaceclofenac (4'-OHACE) and diclofenac (DICLO) (fig. 1).

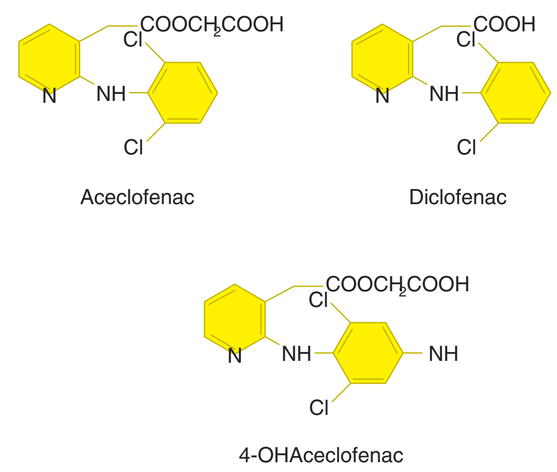

Figure 1.
Structural formulae of aceclofenac, 4'-hydroaceclofenac and diclofenac.

To investigate the mechanisms of action underlying the anti-inflammatory action of ACE, we studied the effect of ACE and its metabolites 4'-OHACE and DICLO on interleukin-6 (IL-6), interleukin-8 (IL-8), nitric oxide (NO) and prostaglandin E_2 (PGE_2) production by human osteoarthritic and normal chondrocytes. All these mediators contribute to the development of chronic synovium inflammation, and then, their inhibition constitutes an interesting therapeutic focal point.

Materials and Methods.

Cartilage specimens were obtained from the knee of 10 donors shortly after death. Upon dissection, femoral and patellar articular surfaces were evaluated for the severity of the macroscopic cartilage lesions. Four out of the ten cartilage samples showed severe lesions (OA cartilage) and six had no macroscopic lesions (normal cartilage).
Enzymatically isolated human chondrocytes were cultured for 72 h in the absence (basal condition) or presence of interleukin-1ß (IL-1ß) (5 U/ml) or lipopolysacharride (LPS) (10 µg/ml) and with or without increased amounts (1 to 30 µM) of ACE or metabolites.
The production of different cytokines and PGE_2 were directly quantified in the culture conditioned medium by specific immunoassays.
Nitrite and nitrate concentrations in the culture supernatants were determined by 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).

Results.

1) Effects of ACE and metabolites on cytokines production by human articular chondrocytes

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.

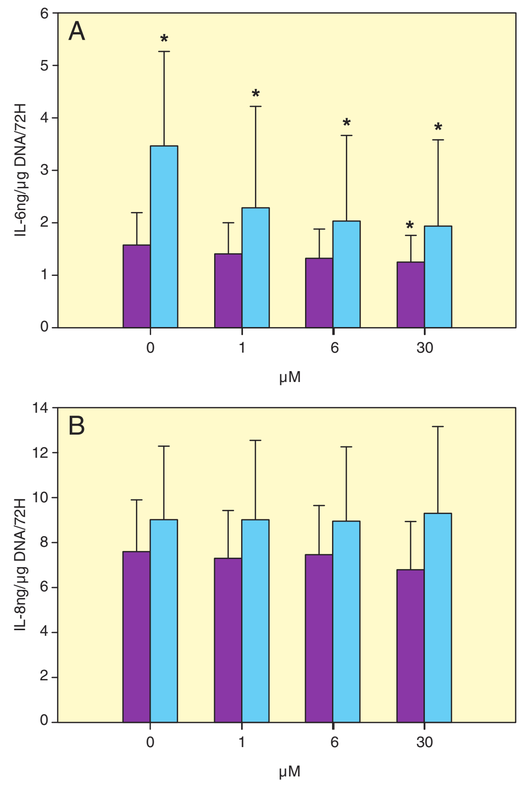

Figure2.
Effects of increased doses of ACE on IL-6 (A) and IL-8 (B) production by human N (green column) or OA (blue column) chondrocytes. Chondrocytes were cultured for 72 h in the presence of IL-1ß (5 U/ml). The data are presented as the mean ± SEM of four different chondrocyte cultures. Each culture was performed with chondrocytes isolated from a single cartilage sample. NSAIDS-treated groups were significantly different from the controls (IL-1ß treated culture) with *p < 0.05.

We have also compared the effect of ACE on IL-6 production with those of its metabolites. Both 4'-HOACE and DICLO were potent inhibitors of IL-1ß-stimulated IL-6 production (fig. 3). At the concentrations of 30 µM, 4'-HOACE and DICLO were significantly more effective than the parent molecule.

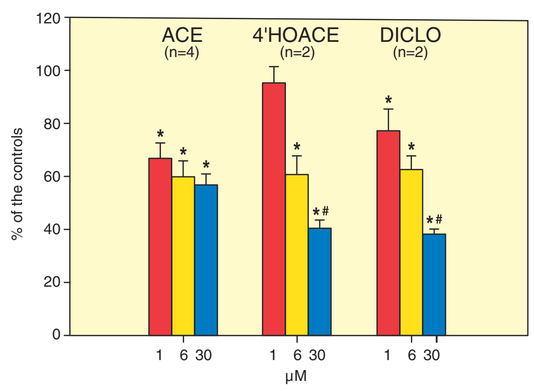

Figure 3.
Effect of increased doses of ACE, DICLO and 4'-HOACE on IL-6 production by human OA chondrocytes cultured for 72 h in the presence of IL-1ß (5 U/ml). The data are expressed as the % of the control values and are presented as the mean ± SEM. NSAIDS-treated groups were significantly different from the controls with *p < 0.05. 4'-HOACE and DICLO inhibitory effects were significantly more important than ACE effect with a # p < 0.05.

ACE and DICLO were ineffective on IL-8 production while 4'-HOACE depressed both basal and IL-1ß-stimulated productions at the concentration of 30 µM.

2) Effects of ACE and metabolites on PGE_2 and NO production by human chondrocytes.

ACE decreased in a dose-dependent manner both basal and IL-1ß-stimulated PGE_2 production. DICLO and 4'-HOACE fully inhibited PGE_2 at all concentrations tested (data not shown).
ACE did not significantly modify NO production (fig. 4). On the other hand, at 30 µM, 4'-HOACE decreased the LPS and the IL-1ß -stimulation (fig. 4). At 30 µM, DICLO inhibited the LPS-stimulated production but was without significant effect on IL-1ß-stimulation.

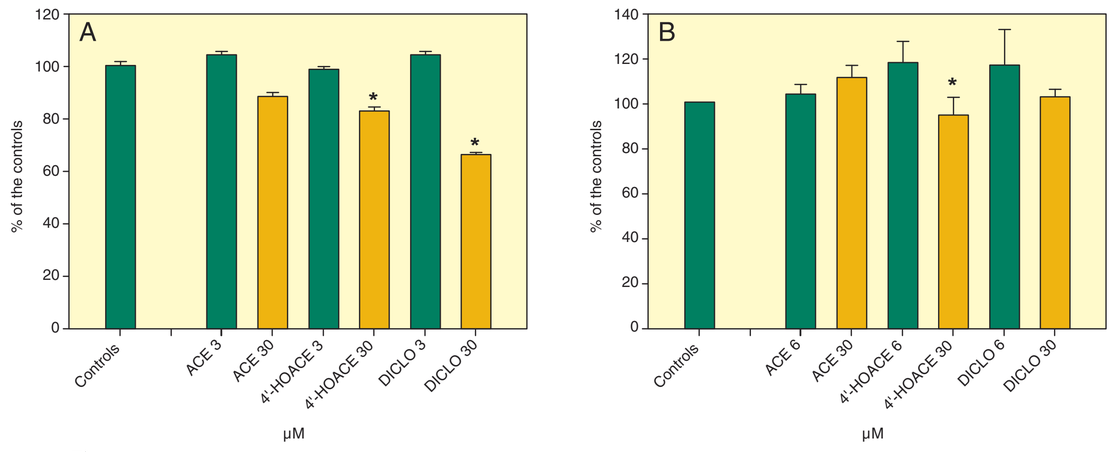

Figure 4.
Effect of increased doses of ACE, DICLO and 4'-HOACE on NO production by human OA chondrocytes. Chondrocytes were untreated (controls) or incubated with LPS (10 µg/ml) (A) or IL-1ß (5 U/ml) (B) alone or in combinaison with ACE, DICLO and 4'-HOACE. Results are expressed as the percentage of the LPS- or IL-1ß-stimulated values (controls) and as the mean ± SEM of two different cultures. NSAIDS-treated groups were significantly different from the controls with *p < 0.05.

2) Effects of ACE and metabolites on iNOS, IL-1ß, COX-2 gene expressions.

LPS induced a marked increase of the iNOS, IL-1ß and COX-2 expression.
At the concentration of 30 µM, 4'-HOACE and DICLO significantly decreased iNOS mRNA, whereas ACE was without effect (fig. 5).
At the concentration of 30 µM, all drugs tested significantly decreased IL-1ß mRNA. At 3 µM, only DICLO was efficient.
ACE and DICLO at the concentration of 30 µM significantly depressed COX-2 mRNA level whereas 4'-HOACE had no effect.

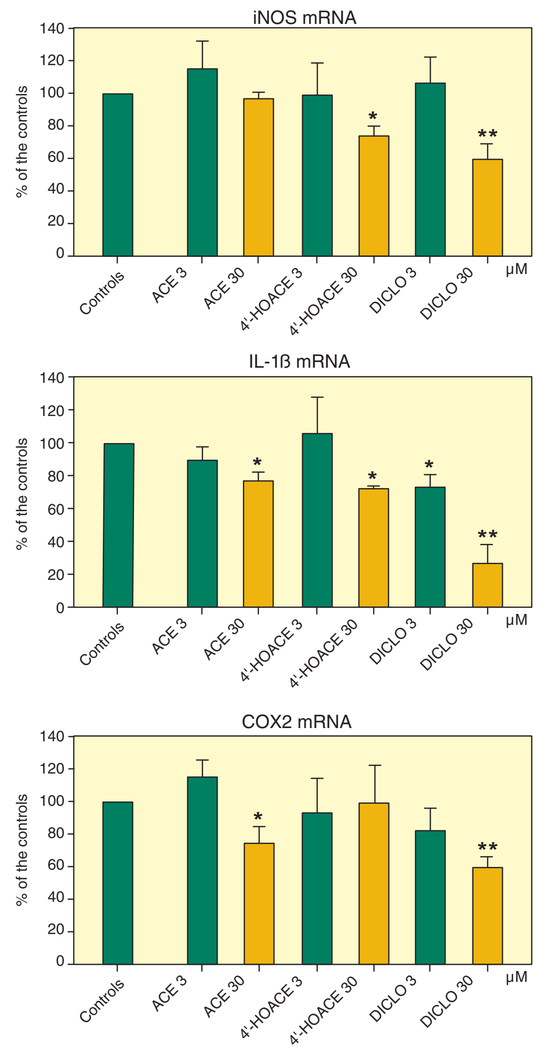

Figure 5.
INOS, IL-1ß and COX-2 mRNA levels were assayed by a real time and quantitative PCR. Results are expressed as the percentage of the LPS-stimulated values (controls). NSAIDS-treated groups were significantly different from the controls with p < 0.05.

Discussion

1) ACE and its metabolites reduce IL-6 synthesis
2) Like ACE, 4'-OHACE and DICLO are potent inhibitors of PGE_2 synthesis
3) ACE does not modify NO production. 4'-HOACE and DICLO inhibit the LPS-stimulated NO production and iNOS gene expression
3) The LPS-stimulated expression of IL-1b is decreased by all drugs

In conclusion, these results suggest that ACE actions are multifactorial and that metabolites could contribute to its anti-inflammatory actions.