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

M Mathy-Hartet (1), A Moulyths-Mickalad (2), G Deby-Dupont (2), Y Henrotin (1,2)
(1) Bone and Cartilage Metabolism Research, Institute of Pathology, CHU, University of Liège, Belgium.
(2) Centre for Oxygen Research and Development (CORD), Institute of Chemistry, University of Liège, Belgium.

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 microsme to form 4'-hydroxyaceclofenac (4'-HOACE) and diclofenac (DICLO) (fig. 1).

Figure 1. Structural formulas of aceclofenac, 4'-hydroxyaceclofenac 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'-HOACE and DICLO on interleukin-6 (IL-6), interleukin-8 (IL-8), nitric oxide (NO) and prostaglandin E2 (PGE2) production by human osteoarthritic and normal chondrocytes. All these mediators contribute to the development of chronic synovium inflammation, and 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 cartilage lesions. Enzymatically isolated human chondrocytes were cultured for the severity of the joint diseases. In healthy human, ACE is rapidly metabolised in the Griess reaction. The production of different cytokines and PGE2 were directly quantified in the culture conditioned medium by specific quantitative PCR. Results are expressed as the percentage of the LPS- or IL-1β-stimulated values (controls).

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-6, 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-6 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 ACE and metabolites on NO production by human OA chondrocytes

ACE did not significantly modify NO production (fig. 4). On the other hand, at 30 µM, 4'-HOACE decreased the LPS and the IL-8-stimulated (fig. 4). At 30 µM, DICLO inhibited the LPS-stimulated production but was without significant effect on IL-8-stimulation.

Figure 2. Effect 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 LPS (5 µg/ml) and with or without increased amounts (1 to 30 µM) of ACE or its metabolites.

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-8-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-8 (5 µg/ml) with or without increased amounts of ACE or its metabolites. Results are expressed as the percentage of the LPS-stimulated values (controls).

Figure 4. Effect of increased doses of ACE, DICLO and 4'-HOACE on NO production by human OA chondrocytes. Chondrocytes were cultured for 3 h in the presence of LPS (5 µg/ml) and with or without increased amounts (1 to 30 µM) of 4'-HOACE, DICLO and 4'-OHAce.

Discussion

ACE and its metabolites reduce IL-6 synthesis. Like ACE, 4'-HOACE and DICLO are potent inhibitors of PGE2 synthesis. ACE does not modify NO production. 4'-HOACE and DICLO inhibit the LPS-stimulated NO production and INOS gene expression.

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