Life Sciences 91 (2012) 1229–1235

Contents lists available at SciVerse ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Further evidence for the anti-inflammatory activity of oleocanthal: Inhibition of MIP-1α and IL-6 in J774 macrophages and in ATDC5 chondrocytes

Morena Scotece [a], Rodolfo Gómez [a], Javier Conde [a], Verónica Lopez [a], Juan J. Gómez-Reino [a], Francisca Lago [b], Amos B. Smith III [c], Oreste Gualillo [a,*]

[a] SERGAS, Santiago University Clinical Hospital Research Laboratory 9 (NEIRID LAB: Neuroendocrine Interactions in Rheumatology and Inflammatory Diseases), Institute of Medical Research (IDIS), Santiago de Compostela 15706, Spain
[b] SERGAS, Santiago University Clinical Hospital, Research Laboratory 7 (Molecular and Cellular Cardiology), Institute of Medical Research (IDIS), Santiago de Compostela 15706, Spain
[c] Department of Chemistry, Monell Chemical Senses Center, and Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia, PA 19104–6323, USA

ARTICLE INFO

Article history:
Received 18 April 2012
Accepted 19 September 2012

Keywords:
Chondrocytes
Macrophages
Nutraceuticals
Natural drugs
Inflammation
Chemokines
Osteoarthritis

ABSTRACT

Aims: Given the relevance of degenerative joint diseases in our society, the development of a novel pharmacologic intervention is a critically important public health goal. Recently, oleocanthal, a polyphenolic natural compound from extra virgin olive oil, has emerged as a potential therapeutic weapon for the treatment of inflammatory degenerative diseases. The goal of this study was to further evaluate the anti-inflammatory activity of oleocanthal in murine macrophages J774 and murine chondrocytes ATDC5 with a particular focus on the inhibition of gene expression of pro-inflammatory factors such as MIP-1α and IL-6.
Main methods: ATDC5 murine chondrogenic cells and murine macrophages J774 were used. J774 macrophages were tested with different doses of oleocanthal and cell viability was evaluated using the MTT assay. Western blot analysis was carried on in J774 cells using anti NOS2 antibody. Nitrite accumulation was determined in culture supernatant using the Griess reaction. MIP-1α and IL-6 mRNA levels were determined using SYBR Green-based quantitative RT-PCR. MIP-1α and IL-6 protein levels were evaluated using specific ELISA assay. Several cytokines, involved in the inflammatory response, were also tested by BioPlex assay.
Key findings: First, oleocanthal inhibits LPS-induced NO production in J774 macrophages, without affecting cell viability. Moreover, it inhibits MIP-1α and IL-6 mRNA expression, as well as protein synthesis, in both ATDC5 chondrocytes and J774 macrophages. Oleocanthal also inhibits IL-1β, TNF-α and GM-CSF protein synthesis from LPS-stimulated macrophages.
Significance: Our data confirm a clear potent role of oleocanthal as anti-inflammatory therapeutic agent for future treatment of arthritis or other inflammatory diseases.

Introduction

Rheumatic diseases are conditions and functional disorders of the musculoskeletal system of non-traumatic causes. They encompass a wide spectrum of conditions, from those of acute onset and short duration to chronic progressive course disorders including osteoarthritis, rheumatoid arthritis, and others. Osteoarthritis (OA) ranks among the major causes of physical disability of elderly patients, thus representing a critical factor in health economics. In contrast to rheumatoid arthritis (RA), OA is conventionally not considered a classical inflammatory arthropathy, but thought to develop from chronic overuse or injury of the joint. However, evidence has accumulated that, besides mechanical and genetic factors, inflammatory processes within joint tissues contribute to the OA onset and progression. Chondrocytes, the unique cell component of articular cartilage, are embedded in a highly organized extracellular matrix (ECM), comprising collagen type II fibrils and proteoglycans, which confer to the cartilage structural rigidity and protective resiliency (Goldring and Marcu, 2009). In response to mechanical or biochemical stress, chondrocytes overexpress pro-inflammatory mediators including cytokines, chemokines and adipokines that will, in an autocrine/paracrine manner, stimulate their own production and induce the expression of matrix-degrading proteinases, including matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) (Bonnet and Walsh, 2005; Goldring and Goldring, 2007; Gomez et al., 2011). In addition to chondrocytes, macrophages that have infiltrated the OA synovium contribute to inflammation and matrix degradation in OA tissues (Benito et al., 2005; Bondeson et al., 2006). Therefore, inflammatory mediators represent potential targets for OA disease modification. Actually, despite some disagreement in the literature, the majority of available studies provide compelling evidence that synovial inflammation is a rationale target for therapeutic intervention to

* Corresponding author. Tel./fax: +34 981950905.
E-mail address: oreste.gualillo@sergas.es (O. Gualillo).

0024-3205/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2012.09.012

control joint symptoms in OA (Scanzello and Goldring, 2012) . On the other side, RA is a common disabling autoimmune disease in the developed world, although the main responsible autoantigen is still unknown. RA disease progression is characterized by chronic erosive inflammation of the joints with invasive proliferation of synovial cells into the articular cartilage and subsequent bone destruction.

Toll-like receptors (TLRs) are phylogenetically conserved receptors involved in the innate immune response. The mammalian homologs of TLRs belong to a family that currently consists of 10 members in humans (Janeway and Medzhitov, 2002). The ligands for several of the TLRs have been identified and include nonbacterial products, such as Hsp70 and fatty acids, as well as microbial constituents such as LPS (O'Neill, 2004). Because ligand recognition by TLRs elicits strong activation of pro-inflammatory cytokines and up-regulation of costimulatory molecules (Bowie and O'Neill, 2000), the role of TLRs in the exacerbation of the inflammatory response and joint destruction in arthritis has been postulated (Kim et al., 2006).

While in osteoarthritis (OA) biomechanical stimuli predominate, with up-regulation of both catabolic and anabolic cytokines and recapitulation of developmental phenotypes, in rheumatoid arthritis inflammation and catabolism drive cartilage loss. In vitro studies have elucidated signaling pathways and transcription factors that orchestrate specific functions that promote cartilage damage in both OA and RA. Among multiple mediators of inflammation in rheumatic disease, MIP-1α and IL-6 have been connected with both OA and RA. While IL-6 at present is widely recognized as relevant mediator of inflammation in rheumatic disease (Tanaka et al., 2011, 2012), the role of MIP-1α is only recently emerging (Iwamoto et al., 2008).

Although the mechanism of cartilage degradation in OA is known to be a multifactorial process, standard pharmacological interventions, such as the use of nonsteroidal anti-inflammatory drugs (NSAIDs), often act in a monomodal way that is frequently associated with significant adverse effects (Fendrick and Greenberg, 2009). Although considerable progress has been made in the development of novel strategies, such as the use of direct MMP inhibitors, no clinically effective inhibitor exists to date. Thus, novel, safe and effective anti-inflammatory agents are demanded for the therapy of arthritic diseases. Nutraceuticals and phytopharmaceuticals that usually contain a range of active compounds targeting multiple pathways could provide an alternative to conventional treatment of OA (Ameye and Chee, 2006).

Indeed, many phenolic compounds extracted from extra virgin olive oil have recently attracted considerable attention, given their antioxidant (Ferroni et al., 2004), anti-inflammatory (Maiuri et al., 2005), and antithrombotic activities (Petroni et al., 1995). In addition, olive oil has been suggested to alleviate a variety of disorders, including cognitive decline due to neurodegeneration (i.e., Alzheimer's disease) (Ramassamy, 2006). In 2005, Beauchamp et al. (2005) isolated and identified an olive oil phenolic compound, (−)-decarboxymethyl ligstroside aglycone (Fig. 1 shows the chemical structure of the compound), also known as oleocanthal (OC) (oleo for olive, canth for sting, and al for aldehyde). Subsequently, Smith et al. (2007) completed the first total synthesis of (−)– oleocanthal and other analogues. Similarly to NSAIDs, oleocanthal induces a strong stinging sensation in the throat and has a potency and pharmacodynamic profile strikingly similar to that of ibuprofen; both compounds inhibit the cyclooxygenase 1 (COX-1) and COX-2 enzymes (Beauchamp et al., 2005). In an earlier study, we extensively examined the anti-inflammatory activity of oleocanthal and several of its derivatives on the expression of nitric oxide synthase type II in chondrocytes (Iacono et al., 2010).

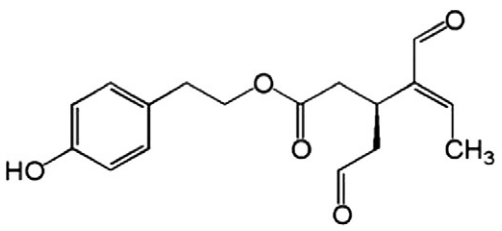

Fig. 1. Chemical structure of 2-(4-hydroxyphenyl) ethyl (3*S*, 4*E*)-4-formyl-3-(2-oxoethyl)hex-4enoate (oleocanthal).

The present study, therefore, aimed to elucidate further the anti-inflammatory activity of oleocanthal in lipopolysaccharide (LPS)-stimulated murine macrophages J774 and ATDC5 human chondrocytes. Particular focus was on the inhibition of pro-inflammatory factors such as MIP-1α and IL-6, and on the interference with nitric oxide (NO) production.

Materials and methods

Chemical and reagents

Fetal bovine serum (FBS), LPS (*E. coli* serotype O55:B5), human transferrin, sodium selenite, and MTT dye were purchased from Sigma. Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium, Dulbecco's modified Eagle's medium (DMEM), trypsin–EDTA, Hepes buffer, sodium pyruvate, L-glutamine and antibiotics were purchased from Lonza.

Cell culture

The murine macrophage J774A.1 cell line was cultured in 75-cm^2 flasks in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, and 130 μg/ml Na pyruvate at 37 °C under 5% CO_2 humidified air.

ATDC5 cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium supplemented with 5% FBS, 10 μg/ml recombinant human insulin, 10 μg/ml human transferring, 3×10^{-8} M sodium selenite, and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin).

Cell viability

Cell viability was examined as previously described (Iacono et al., 2010). Briefly, using a colorimetric assay based on the MTT labeling reagent, J774 cells (25×10^3/well) were seeded in 96-well plates. Briefly, cells were stimulated with oleocanthal (10–100 μM) in 10% FBS medium for 48 h at 37 °C. After that, cells were incubated with 10 μl of MTT (5 mg/ml) for 4 h at 37 °C. Then, after dissolving the formazan salt, the spectrophotometric absorbance was measured using a microtiter enzyme-linked immunosorbent assay reader at 550 nm (Multiskan EX; Thermo Labsystems).

Cell treatments and nitrite assay

J774 were plated at density of 5×10^5 cells/well in 6-well plates. After complete adhesion and 4 h of starvation in serum-free medium, cells were pre-incubated with oleocanthal 50 μM for 12 h at 37 °C and then stimulated with LPS (250 ng/ml) in 10% FBS medium for 24 h at 37 °C.

ATDC5 were seeded in 6-well plates at 2.5×10^5 cells/well. After 24 h of starvation in serum-free medium, cells were pretreated with oleocanthal 15 μM for 12 h at 37 °C and then stimulated with LPS for 24 h at 37 °C in 5% FBS medium.

Nitrite accumulation was measured in culture medium using the Griess reaction as previously described (Otero et al., 2005). Briefly, 100 μl cell culture medium was mixed with 100 μl Griess reagent (equal volumes of 1% [weight/vol] sulfanilamide in 5% [vol/vol] phosphoric acid and 0.1% [weight/vol] naphtylethylenediamine-HCl), incubated at room temperature for 10 min, and then the absorbance at 550 nm was measured using a microplate reader (Titertek-Multiscan,