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Original Paper

Oleocanthal Inhibits Catabolic and Inflammatory Mediators in LPS-Activated Human Primary Osteoarthritis (OA) Chondrocytes Through MAPKs/NF-κB Pathways

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Key Words
Oleocanthal • Chondrocytes • Phenolic component • Inflammation • Osteoarthritis

Abstract
Background/Aims: Oleocanthal (OC), a phenolic compound present in extra virgin olive oil (EVOO), has attracted attention since its discovery for its relevant pharmacological properties in different pathogenic processes, including inflammation. Here, we investigated the involvement of OC in LPS-activated osteoarthritis (OA) human primary chondrocytes. ***Methods:*** Human primary chondrocytes were harvested from articular cartilage samples obtained from OA patients. The effects of OC on the viability of chondrocytes were tested by MTT assay. Protein and mRNA expression of several catabolic and pro-inflammatory factors after OC treatment were measured by RT-qPCR and western blot respectively. Moreover, we analysed the NO production by Griess reaction. Finally, several pathways mediators were analysed by western blot. ***Results:*** We demonstrated that OC did not have any cytotoxic effect. Oleocanthal inhibited NO production and strongly decreased NOS2 and COX-2 protein and mRNA expression in

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LPS-activated human primary OA chondrocytes. Interestingly, OC also inhibits MMP-13 and ADAMTS-5. In addition, OC downregulates several pro-inflammatory factors, such as IL-6, IL-8, CCL3, LCN2 and TNF-α induced by LPS in human primary OA chondrocytes. Finally, we demonstrated that OC exerts its effects through the MAPK/P38/NF-κB pathways. ***Conclusion:*** These data show that OC is able to block LPS-mediated inflammatory response and MMP-13 and ADAMTS-5 induction in human primary OA chondrocytes via MAPKs/NF-κB pathways, suggesting that OC may be a promising agent for the treatment of inflammation in cartilage and a potential molecule to prevent disease progression by inhibiting metalloproteases and aggrecanases.

Introduction

Osteoarthritis (OA) is a multifactorial degenerative muscle-skeletal disease with no curative treatment. OA is characterized by a low-grade inflammation and deep morphological changes in cartilage as well as in subchondral bone. Cartilage fragmentation is the cause of synovial inflammation that is now recognised as a key process of OA joint pathology. OA aetiology is not fully understood. However, it has been suggested that chondrocytes inflammatory responses could mediate OA in terms of development and progression [1]. One of the factors involved in these responses is IL-1, a cytokine proposed as a driver of OA disease [2, 3]. Nevertheless, therapeutics attempts driven to target IL-1 are not able to control OA symptoms [4].

In light of this, there is a growing interest in studying other potential targets, in order to develop new therapeutic approaches to halt OA progression. Recently, Toll-like receptors (TLRs) are emerging as potential new targets for OA [5–7]. High levels of TLRs agonists are present in OA joints [8] and TLRs have been associated with OA [9, 10]. Particularly, it has been reported that TLR4 is associated with OA mediating cytokines production and inflammatory responses in chondrocytes [8]. TLR4 binds a number of different agonists, for example, the pathogen-associated molecular patterns (PAMPs), such as LPS, but also recognize damage-associated molecular patterns (DAMPs), some of which are released when tissues are damaged [11].

Several molecules involved in TLR4 activation have an important role in cartilage inflammatory responses and in the development and progression of cartilage degradation. There is increasing evidence that nitric oxide (NO) is one of the factors induced by TLR4 activation and its excessive production is associated with OA progression [12]. NO is a short-lived gaseous mediator of inflammatory response mainly produced by nitric oxide synthase (NOS2) and highly produced by chondrocytes upon pro-inflammatory cytokine stimulation [13, 14]. This pleiotropic free radical regulates the synthesis of several catabolic enzymes like matrix metalloproteinases (MMPs), which play a key role in the OA-associated cartilage degradation through the induction of the breakdown of cartilage matrix components [15].

Several cytokines and chemokines are also associated with TLR4 activation and are involved in cartilage inflammatory responses leading to the development and progression of cartilage degradation. For instance, TNF-α, IL-6 and IL-8 have been implicated in OA progression [16] as well as chemokine MIP-1α [17].

Current treatment options to manage OA are not curative and fail to reverse the degenerative process of OA. Thus, the need for effective OA treatment, with lower side effects than those exerted by NSAIDs or analgesic drugs such as acetaminophen, is of particular relevance.

Vegetal–derived compounds show relevant pharmacological properties that can be explored in the context of cartilage pathophysiology to identify novel molecules as well novel pharmacological properties of natural compounds. Oleocanthal (OC) is a phenolic component of extra virgin olive oil (EVOO), the main lipid component of the Mediterranean diet. OC (*oleo*-for olive, *canth*-for sting, and *–al* for aldehyde) is responsible for the throat burn characteristic of many EVOOs [18–22].

OC was described to inhibit COXs in cell-free enzymatic assay [19] in a way similar to well-known no steroidal anti-inflammatory drug (NSAID) used in the standard pharmacological intervention of OA.

Several studies evidenced novel relevant pharmacological properties of OC in different pathogenic processes. Our group elucidated the anti-inflammatory properties of oleocanthal in LPS-activated murine macrophages J774 and ATDC5 murine chondrogenic cells [23, 24]. In addition, we demonstrated a potent *in vitro* anti proliferative mechanism exerted by oleocanthal in human and mouse multiple myeloma cells [25]. This anti-cancer activity of OC was confirmed by several other studies [26–29]. OC is also associated with a reduced risk of neurodegenerative diseases, such as Alzheimer's disease [30–32]. However, nothing is known about the effect of OC in human OA pathology.

Therefore, the current study aimed at identifying the effect of OC in LPS-stimulated human primary chondrocytes from OA patients. Moreover, we investigate the signalling pathway involved in this process, focusing on the activation of ERK 1/2, P38 and the IκB/NF-κB pathway.

Materials and Methods

Reagents

Fetal bovine serum (FBS), LPS (*E coli* serotype O55:B5) and MTT dye were purchased from Sigma. Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium, trypsin–EDTA, l-glutamine and antibiotics were purchased from Lonza. Pronase and Collagenase P were obtained from Roche Molecular Biochemicals.

Cartilage samples, and chondrocytes isolation and culture.

Primary chondrocytes were harvested from human OA articular cartilage samples obtained from articular joints of patients undergoing total knee replacement surgery at the Orthopaedic Division of the Xerencia de Xestión Integrada de Santiago de Compostela, Spain. All the procedures have been approved by the Galicia Clinical Studies Ethical Committee (Comité Autonómico de Ética da Investigación de Galicia Secretaria Xeral. Consellería de Sanidade Edificio Administrativo San Lázaro 15703 SANTIAGO DE COMPOSTELA). NºReg. 2014/310.

For chondrocyte isolation, aseptically dissected cartilage was subjected to sequential digestion with pronase and collagenase P at a final concentration of 1 mg/ml in Dulbecco's modified Eagle's medium/F12 plus 10% fetal calf serum and sterilized by filtration, in accordance with the manufacturer's instructions. Cartilage specimens were finely diced in phosphate-buffered saline (PBS), and after removing PBS diced tissue was incubated for 30 min with pronase at 37°C. Pronase was subsequently removed from the digestion flask and the cartilage pieces were washed with PBS. After removal of PBS, digestion was continued with the addition of collagenase P; this was done over 6–8 hours in a shaking water bath at 37°C. The resulting cell suspension was filtered through a 40 μm nylon cell strainer (BD Biosciences Europe, Erembodegem, Belgium) in order to remove debris. Cells were centrifuged and washed one time with PBS and one time with Dulbecco's modified Eagle's medium/F12 plus 10% fetal calf serum, counted and plated in 6-well tissue culture plates for chondrocyte culture. Cells were serially passaged to obtain a sufficient number of cells and used between the first and second passages and plated in 12-well tissue culture plate.

Cell viability

Cell viability was examined using a colorimetric assay based on the MTT labelling reagent. Primary human chondrocytes from OA patients (8000 cell//well) were seeded in 96-well plates. Briefly, cells were stimulated with oleocanthal (1-5 μM) for 12 hours at 37°C and next stimulated with LPS 250 ng/mL during 24 hours. After that, cells were incubated with 10 μl of MTT (5 mg/mL) for 4 hours at 37 °C. Then, after dissolving the formazan, 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

Primary human OA chondrocytes were plated in 12-well plates. After 4 hours of starvation, cells were pre-incubated with oleocanthal 1-5 μM for 12 hours at 37 ^{0}C and then stimulated with LPS (250 ng/mL) for 24 hours at 37 °C.

Nitrite accumulation was measured in culture medium using the Griess reaction. 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, Labsystem, Helsinki, Finland). Fresh culture medium was used as blank in all of the experiments. The amount of nitrite in the samples (in micromolar units) was calculated from a sodium nitrite standard curve freshly prepared in culture medium.

mRNA isolation and RT-qPCR

RNA was isolated from cell culture by Trizol LS, according to the manufacturer's instructions (Gibco BRL Life Technologies, Grand Island, NY).

Human NOS2, human COX2, human MMP-13, human IL-6, human IL-8, human MIP1-α (CCL3), human TNF-α, human LCN2 and human ADAMTS-5 mRNA levels were determined using SYBR Green–based quantitative PCR (qPCR). RNA was extracted using a NucleoSpin kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. For relative quantification, we performed an RT reaction with a Thermo Scientific Verso cDNA Synthesis Kit (42°C for 30 min, followed by an incubation at 95°C for 2 min). RT-qPCR was performed in a Stratagene MX3005P thermal cycler using a standard protocol (95°C for 10 min followed by 40 cycles for 15 s of denaturation at 95°C and 1 min annealing/extension at 60°C), a SABiosciences Master Mix and specific primers (human NOS2, PPH00173E; human COX2 PPH01136F; human LCN2 PPH00446E; human ADAMTS-5 PPH09588A; human MMP-13, PPH00121B; human IL-6, PPH00560B; human IL-8, PPH00568A; human MIP-1α, PPH00566E; human TNF-α, PPH00341F). Results were calculated using the comparative (ΔΔCt) method and the MxPro software (Stratagene, California, USA), as the ratio of each gene to the expression of the housekeeping gene.

Data are shown as mean ± SEM (error bars) of at least three independent experiments and represented as fold-change vs controls. Melting curves were generated to ensure a single gene-specific peak, and no-template controls were included for each run and each set of primers to control for unspecific amplifications.

Cell treatments, protein extraction and western blotting analysis

Primary human OA chondrocytes were plated in 12-well plates, stimulated with oleocanthal 1-5 μM 12 hours and then incubated with LPS (250 ng/mL) during 24 hours or 30 minutes.

Proteins were extracted using a lysis buffer with a commercial protease inhibitor cocktail (Thermo Fisher). SDS-PAGE and blotting procedure were carried on as previously described [13]. Immunoblots were incubated with the appropriate antibody (iNOS, IκB, p-ERK-1/2 and NF-κB p65 from Cell Signaling MA, USA; COX-2 from DAKO, Denmark; MMP13 from Santa Cruz Biotechnology, USA; ERK1/2 and p-38 from Upstate, p-p38 from Millipore; Lamin B1 from Boster, CA, USA) and visualized with an Immobilon Western Detection kit (Millipore Massachusetts, USA) using horseradish peroxidase-labelled secondary antibody. To confirm equal loading in each sample, the membranes were stripped in glycine buffer at pH 2 and re-blotted with an anti-GAPDH antibody (Sigma). The images were captured and analysed with an EC3 imaging system (UVP). Data obtained were further validated by densitometric analysis using Image J software.

Statistical analysis

Data are reported as the mean ± SEM of at least 3 independent experiments. Statistical analysis was performed using analysis of variance followed by Bonferroni multiple comparison test using the Prism computerized package (Graph Pad Software). *p values* less than 0.05 were considered statistically significant.

Results

Cytotoxicity assay: effect of Oleocanthal on the viability of primary human OA chondrocytes

Oleocanthal, the (-)-decarboxymethyl ligstroside aglycone (Fig. 1, panel A), represents approximately 10 % of the total phenolic compounds in extra virgin olive oil (EVOO).

First, we assessed the potential cytotoxicity of OC in human primary OA chondrocytes. As shown in Fig. 1 panel B, the doses of 1 and 5 μM of OC did not alter chondrocyte viability, even in combination with inflammatory stimuli (LPS).

Effect of Oleocanthal on nitric oxide (NO), NOS2 and COX2 production

To test the anti-inflammatory activities of OC, we evaluated the effect of the compound in human OA chondrocytes stimulated with LPS, a TLR4 agonist. First, we analysed the effect of OC on LPS-induced NO production.

As shown in Fig. 2, LPS led to a significant accumulation of NO (panel A) in human OA cell culture supernatant. OC, at 1 and 5 μM, strongly decreased NO production in LPS-activated chondrocytes compared with control cells (Fig. 2, panel A).

To investigate whether the effect of OC on NO accumulation was related to the inhibition of NOS2 expression, we analysed by RT-qPCR the NOS2 mRNA expression (panel B) and the NOS2 protein expression by Western Blotting (panel C). As shown in Fig. 2, the pre-treatment with OC downregulated the NOS2 mRNA expression (panel B) and completely inhibited NOS2 protein expression (panel C) in LPS-activated human primary chondrocytes from OA patients (Fig. 2, panel B and C).

To gain further insight into the anti- inflammatory activity of OC, we studied the effect of this compound also on the cyclooxygenase-2 protein expression, one of the most important enzymes of the inflammatory response responsible for the synthesis of prostaglandins. As shown in Fig. 2, OC was able to significantly inhibit COX-2 mRNA (panel D) and protein (panel E) expression induced by LPS in OA human primary chondrocytes (Fig. 2, panel D and E).

Effect of Oleocanthal on MMP-13 production and ADAMTS-5 mRNA expression

In order to analyse whether OC was also able to inhibit one of the main catabolic factors of the cartilage degradation, we studied the effect on MMP-13 production. As shown in Fig. 3, OC suppressed MMP-13 mRNA (panel A) and protein (panel B) expression after LPS stimulation in primary human OA chondrocytes (Fig. 3).

In addition, we explored the effect of OC on the aggrecanase ADAMTS-5 that mediates aggrecan degradation, a significant event in the early stage of OA.

As shown in Fig. 3, Oleocanthal downregulated the ADAMTS-5 mRNA expression after LPS stimulation in primary human OA chondrocytes (panel C).

Oleoacanthal reduces several pro-inflammatory factors in human primary OA chondrocytes

Several cytokines, chemokines and adipokines are implicated in inflammatory and catabolic OA processes. Here, we explored the ability of OC to modulate some of the most important factors involved in the cartilage degradation process.

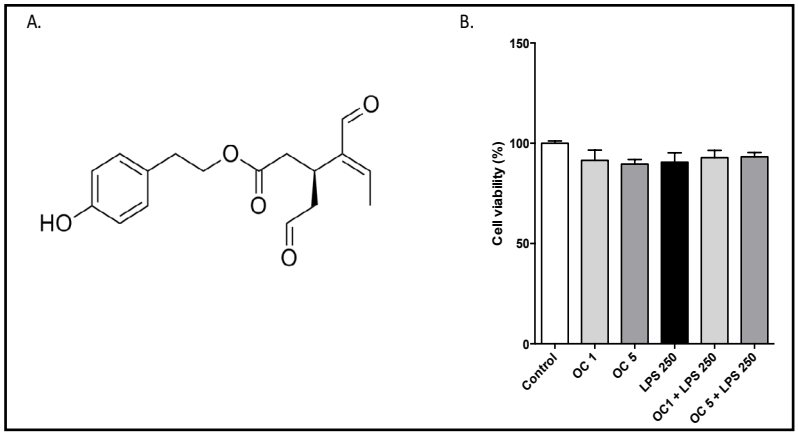

Fig. 1. Effect of Oleocanthal on primary human OA chondrocytes viability. Chemical structure of OC (A). Primary human OA chondrocytes were pre-incubated with OC (1 and 5 μM) during 12 hours and then stimulated with LPS (250 ng/mL) for 24 hours. Subsequently cells were processed to test the effect of OC on cell viability using MTT assay. Experiments were performed at least in triplicate, with eight independent observations for each treatment and experiment (B).

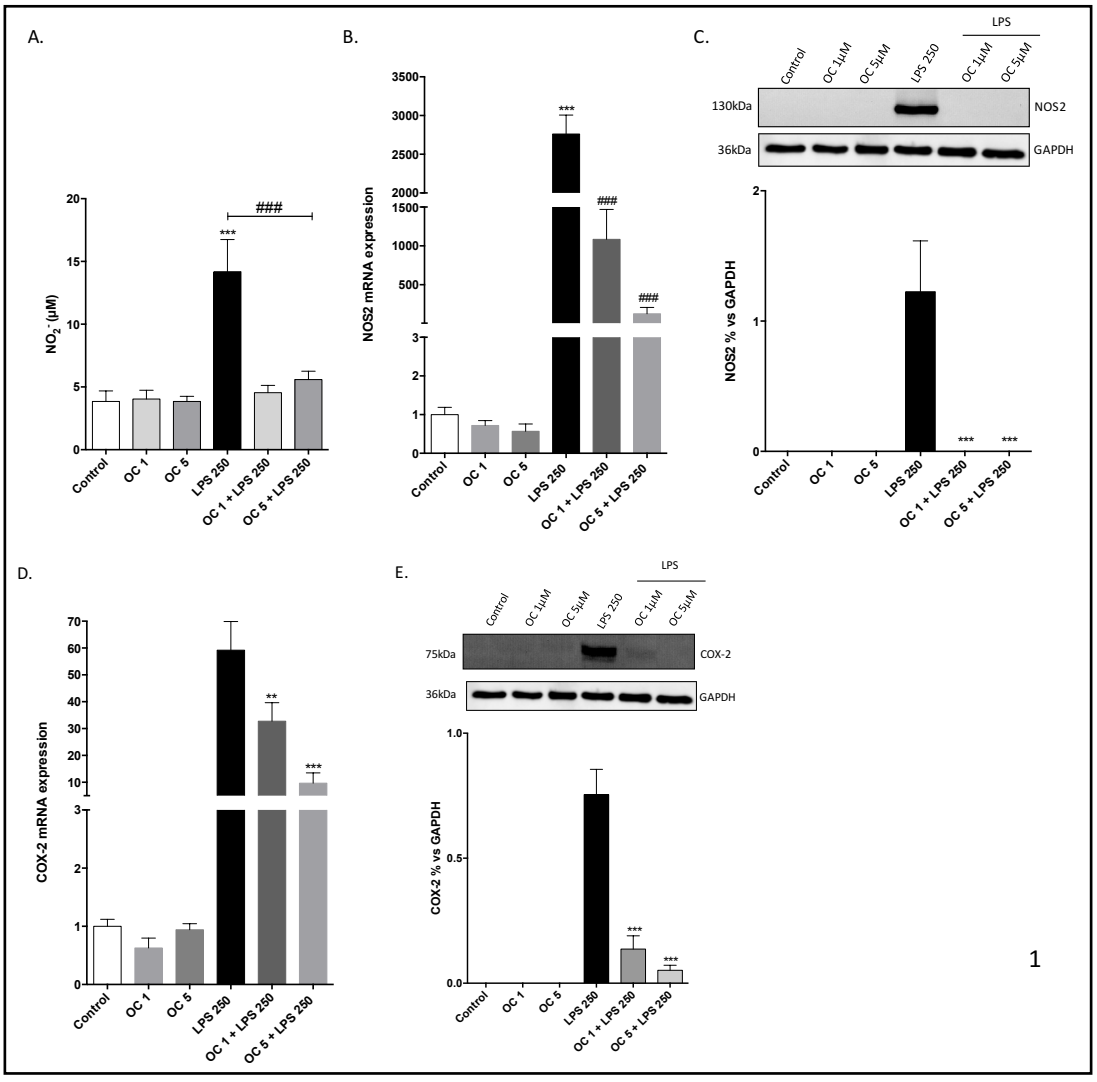

Fig. 2. Modulation of nitric oxide (NO) production, NOS2 and COX2 mRNA and protein expression by OC. Primary human OA chondrocytes are pre-treated for 12 hours with OC (1 and 5 μM) and then stimulated with LPS (250 ng/mL) (panel A, B and C) during 24 hours. NO production was evaluated as nitrite accumulation conditioned media using Griess reaction (A). NOS2 (B) and COX2 (D) mRNA expression was determined by RT-qPCR (B). NOS2 (C) and COX2 (E) protein expression was measured by western blotting (GAPDH was used as internal standard). Results are presented as mean ± SEM of at least three independent experiments. In panel A ### $p<0.001$ versus LPS and *** $p<0.001$ versus unstimulated control; in panel B ### $p<0.001$ versus LPS and *** $p<0.001$ versus unstimulated control; in panel C *** $p<0.001$ versus LPS; in panel D ** $p<0.01$, *** $p<0.001$ versus LPS; in panel E *** $p<0.001$ versus LPS.

As shown in Fig. 4, the production of chemokine MIP-1α (panel A), and cytokines IL-8 (panel B), IL-6 (panel C) and TNF-α (panel D) by LPS stimulation in human primary OA chondrocytes, was downregulated by OC treatment in a dose dependent manner.

In addition, OC was able to inhibit also LCN2 (panel E), an adipokine implicate in the catabolic mechanisms of OA (Fig. 4).

Oleocanthal inhibits p-ERK1/2, p-P38 and NF-kB signalling pathways

To achieve further insights into the mechanisms of OC activity, we explored the effect of OC on the TLR4 signalling pathway activated by LPS. Since MAPKs are the downstream target of LPS-induced inflammatory cascades, we analysed the MAPK family members phospho

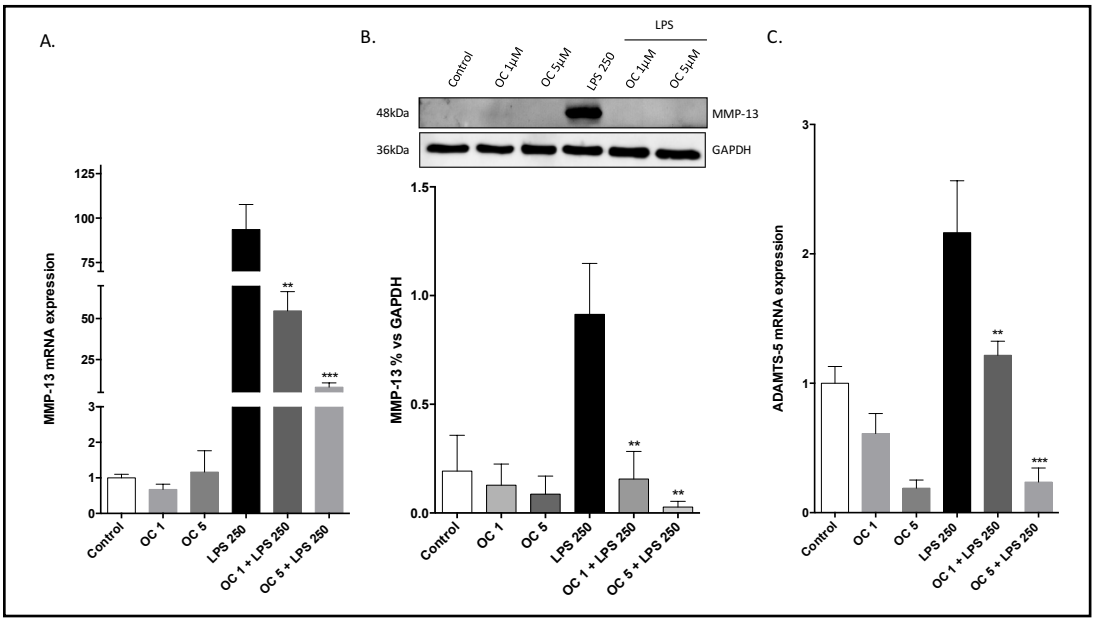

Fig. 3. Oleocanthal modulation on MMP-13 and ADAMTS-5 expression. Western blots were performed with protein lysates from primary human OA chondrocytes pre-treated for 12 hours with OC (1 and 5 μM) and then incubated with LPS (250 ng/mL) (panel B) during 24 hours to detect MMP-13. GAPDH was used as internal standard. RT-qPCR was performed to analyse the MMP13 mRNA (panel A) and ADAMTS-5 expression (panel C). Results are presented as mean ± SEM of at least three independent experiments. **p<0.01, ***p<0.001 versus LPS.

ERK1/2 and phospho p38 by western blotting.

As we can see in Fig. 5, LPS stimulation led to an increase of p-ERK1/2 (panel A) and p-P38 (panel B) which was significantly inhibit by OC treatment (Fig. 5).

To gain additional insights into the mechanism of action underlying the down-regulation of the inflammatory and catabolic factors by OC, we also analysed the effect of OC on IκB by western blotting. As shown in Fig. 5, LPS degraded IκB after 30 minutes of incubation. The addition of OC significantly inhibited the degradation of IκB blocking the translocation of NF-κB to the nucleus with consequent inactivation of the transcription of pro-inflammatory target genes (Fig. 5, panel C). In line with this, in the nucleus, OC decreased the expression of NF-κB subunit p65 induced by LPS stimulation (Fig. 5, panel D).

Discussion

The present study demonstrated for the first time that OC, a phenolic component of extra virgin olive oil (EVOO), strongly reduces inflammatory and catabolic responses in LPS-activated primary human OA chondrocytes.

Extra virgin olive oil (EVOO), the main lipid component of the Mediterranean diet, is associated with many beneficial health activities, including lower incidences of cardiovascular mortality, age-related cognitive disease, and cancer [33–38]. Oleocanthal has attracted considerable attention given its anti-inflammatory, anti-neurodegenerative and anti-cancer activities [20, 23, 32, 24–31]. Our group demonstrated that OC was able to decrease inflammatory mediators in murine macrophages and mouse chondrogenic cells [23, 24], opening the interest to study the potential effect of this phenolic compound in inflammatory degenerative joints diseases, such as OA.

OA, the most common form of arthritis, is the major cause of disability and pain in older adults. Progressive cartilage degradation characterized OA pathogenesis with consequent

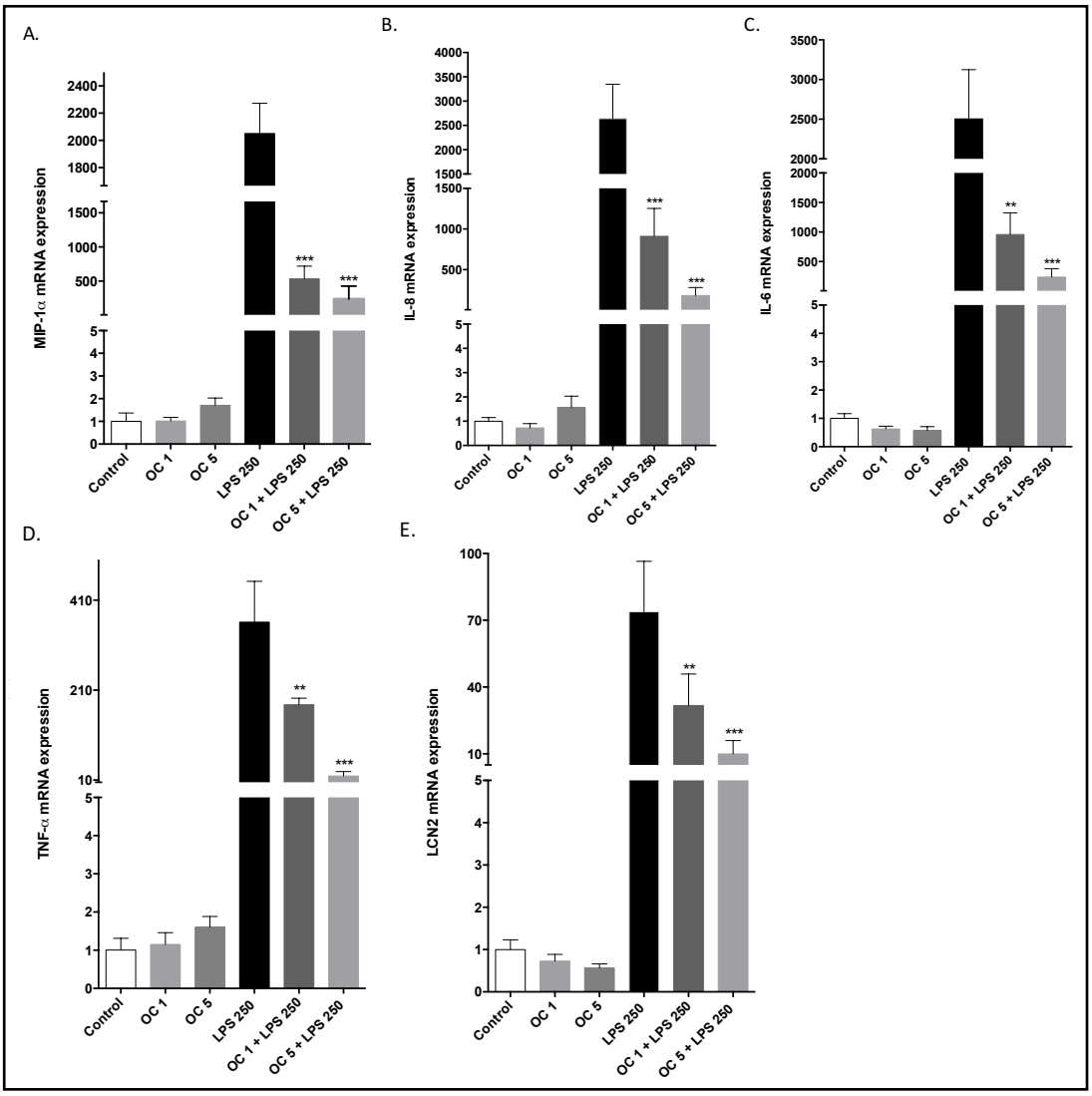

Fig. 4. OC inhibits pro-inflammatory factors in human primary OA chondrocytes. RT-qPCR were performed with RNA extraction from primary human OA chondrocytes pre-treated for 12 hours with OC (1 and 5 μM) and then incubated with LPS (250 ng/mL) during 24 hours to detect MIP1-α (panel A), IL-8 (panel B), IL-6 (panel C), TNF-α (panel D) and LCN2 (panel E) mRNA expression. Results are presented as mean ± SEM of at least three independent experiments. **p<0.01, ***p<0.001 versus LPS.

joint space narrowing. Classical therapeutic interventions in OA fail to modify the progression of the disease and are frequently associated with significant adverse effects [39]. However, since it has been reported that inflammation plays a key role in OA progression [9], new therapeutic strategies have been suggested in order to control the inflammatory processes.

The control of inflammatory responses by blocking IL-1 action, a key factor associated with OA perpetuation, has been recently suggested. However, the inhibition of IL-1 activity failed to control OA symptoms [4], motivating research to find new potential therapeutic approaches. Recently, Toll-like receptors (TLRs) are becoming one of these potential new targets for OA treatment [7], since high levels of TLRs agonists are present in OA joints [8] and TLRs, particularly TLR4, have been associated with OA [8–10].

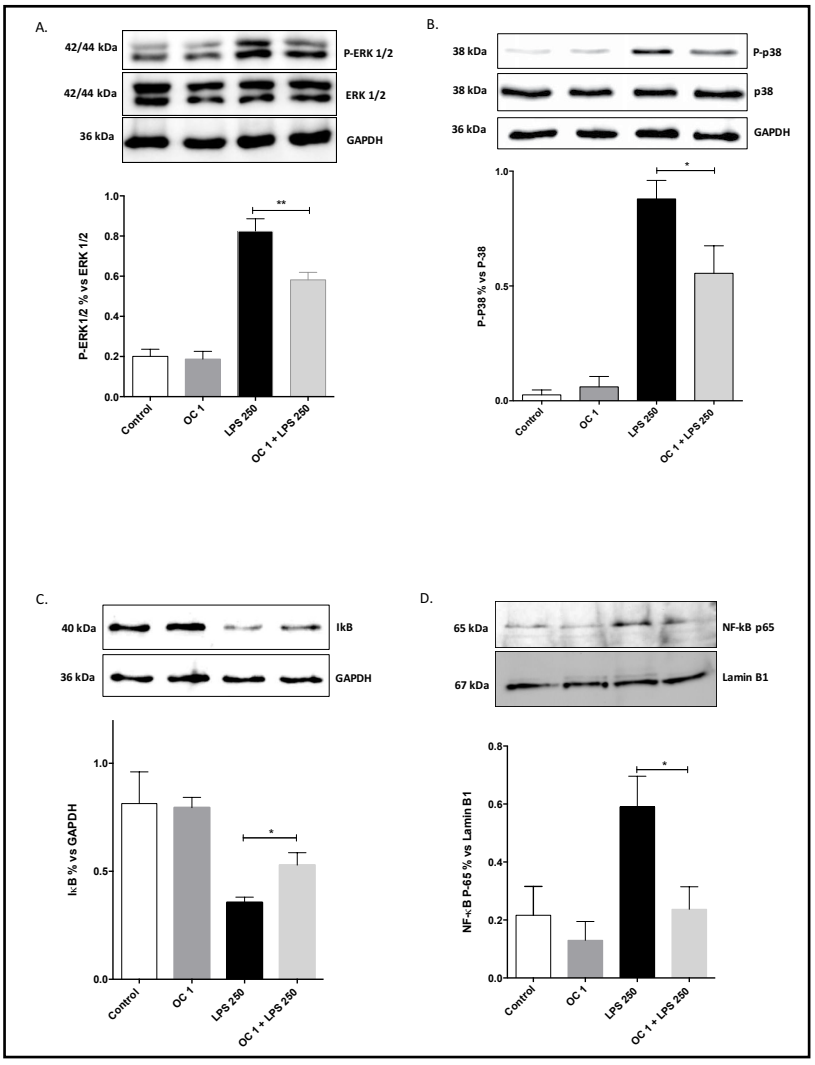

Fig. 5. Molecular mechanism implicated in OC activity in human OA primary chondrocytes. Western blots were performed with protein lysate from human OA primary chondrocytes pre-treated 12 hours with OC (1 μM) and then stimulated 30 minutes with LPS (250 ng/mL) to detect p-ERK1/2 (panel A), p-P38 (panel B) and IκB (panel C). GAPDH was used as internal standard. NF-κB p65 (panel D) expression was determined by western blot using nuclear extract. Lamin B1 was used as internal standard in nuclear protein extract. Results are presented as mean ± SEM of at least three independent experiments. *p<0.05, **p<0.01 versus LPS.

Consequently, we planned to study the effect of OC on inflammatory and catabolic responses in primary human OA chondrocytes stimulated with LPS, a TLR4 agonist.

As for as we are aware, this is the first time that oleocanthal is tested in human primary chondrocytes.

First of all, we analysed the cytotoxic effect of two different doses of OC (1 and 5 μM) in primary human OA chondrocytes. We found that OC didn't have cytotoxic effect in these cells.

Our results are in agreement with those reported by Iacono et al. and by Scotece et al. [23, 24] in macrophages and murine chondrocytes.

Here, we demonstrated that OC strongly decreases NO production and NOS2 protein expression in LPS-stimulated primary human OA chondrocytes.

It is known that NO is a gaseous mediator of the inflammatory response that is involved in multiple inflammatory degenerative diseases, such as OA [12]. The increase of NO in chondrocytes determines the induction of apoptosis, activation of inflammatory responses and matrix degradation. This catabolic factor may reflect the degree of inflammation providing a measure to assess the effect of potential anti-inflammatory drugs on the joint degenerative inflammatory process. Thus, the inhibition of NO production and NOS2 protein expression by OC might contribute in reducing inflammatory and degenerative processes in cartilage. In addition, we found that the protein expression of COX2, one of the most important mediators of the inflammatory response, was strongly inhibited by OC, confirming the anti-inflammatory properties of this compound.

To test the effect on the catabolic response, we analysed the activity of OC on the matrix metalloproteinase MMP-13, which induce the breakdown of cartilage matrix components playing a key role in the OA-associated cartilage degradation [15]. We demonstrated that OC strongly inhibits MMP-13 protein expression in LPS-activated chondrocytes. Moreover, we also explored the activity of OC on ADAMTS-5, one of the most important aggrecanases involved in the progression of OA disease [40].

As mentioned above, inflammation plays a key role in OA pathology. Several studies reported the role of pro-inflammatory factors, such as cytokines, chemokines and adipokines as critical molecules involved in cartilage degradation [16, 41]. Thus, we explored some relevant factors involved in OA. Specifically, we showed that OC inhibits TNF-α, IL-8 and IL-6 mRNA expression, Furthermore, we demonstrated that OC is also able to inhibit the chemokine MIP-1α. In addition, OC downregulated the induction of LCN2 by LPS, suggesting a role for OC also in the modulation of the adipokines which are implicated in chondrocytes and cartilage degradation [42–46].

In order to study the molecular events involved in OC effects in chondrocytes, we performed western blots to detect p-ERK1/2, p-38 IκB and NF-κB. We demonstrated that OC inhibits ERK1/2 and p-38 phosphorylation induced by LPS in human OA primary chondrocytes. On the other side, OC increased the IκB expression leading to the block of NF-κB into the cytosol and decrease the nuclear expression of NF-κB p65 induced by LPS. The inhibition of these pathways led to the impediment of the transcription of inflammatory genes such as IL-6, IL-8, COX-2, NOS-2, MIP-1α, TNF-α, LCN2, MMP13 and ADAMTS-5 (Fig. 6) reducing the inflammatory and catabolic processes in OA chondrocytes.

The data presented above demonstrated for the first time, that OC inhibits inflammatory responses induced by TLR4 activation in primary human OA chondrocytes, suggesting a potential use of this compound in OA treatment. Furthermore, we also showed its anti-catabolic activity, both indirect, inhibiting NO production and direct, acting on MMP13 and ADMTS-5. Finally, we demonstrated that the anti-inflammatory and anti-catabolic effects of OC in human primary OA chondrocytes were mediated, at least in part, by blocking MAPKs/NF-κB pathways.

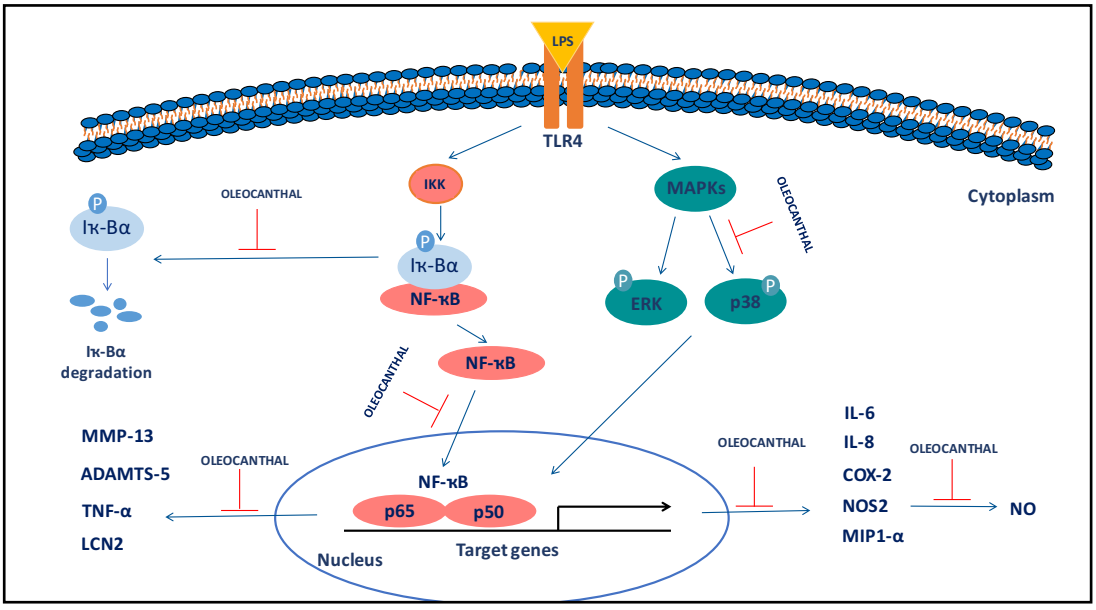

Fig. 6. Schematic representation of OC effects in human primary OA chondrocytes. The inhibition of MAPKs/NF-κB pathways mediates the effects of OC in human primary OA chondrocytes. Inflammatory and catabolic responses induced by TLR4 activation in primary human OA chondrocytes are inhibited by OC through the downregulation of several inflammatory factors such as cytokines (IL-6, IL-8, TNF-α), COX-2, NOS2, chemokines (MIP1-α), adipokines (LCN2), NO, and pro-catabolic factors like MMP-13 and ADAMTS-5.

Conclusion

Our study demonstrates that OC is able to inhibit inflammatory and catabolic responses in LPS-activated OA chondrocytes suggesting a potential use of this phenol against cartilage degradation process in OA. Evidently, data reported in this article are limited to an *in vitro* study. Thus, further experiments are needed to test and verify the effects of OC *in vivo* using animal models. Anyway, here we provide, for the first time, clear evidence on the inhibition by OC of inflammatory and catabolic processes in OA human chondrocytes, opening novel potential approaches for OA treatment.

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Disclosure Statement

The authors declare no conflict of interests.

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