Benefit of combining curcumin, harpagophytum and bromelain to reduce inflammation in osteoarthritic synovial cells

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Abstract

Background: Osteoarthritis is the most common cause of arthritis affecting millions of people worldwide, characterized by joint pain and inflammation. It is a complex disease involving inflammatory factors and affecting the whole joint including synovium. Since drug combination is widely used to treat chronic inflammatory diseases, a similar strategy may be worth of interest to design plant-derived natural products to reduce inflammation in OA joint. Here, we characterized the response of OA synovial cells to lipopolysaccharide (LPS) and investigated the biological action of the combination of curcumin, harpagophytum and bromelain in this original *in vitro* model of osteoarthritis.

Methods: Primary, human synovial cells from OA patients were stimulated with LPS and proteomic analysis was performed. Bioinformatics analysis were performed using Cytoscape App and SkeletalVis databases. Additionally, cells were treated with curcumin, harpagophytum and bromelain alone or the three vegetal compounds together. The expression of genes involved in inflammation, pain or catabolism were determined by RT-PCR. The release of the encoded proteins by these genes and of prostaglandin E2 (PGE2) were also assayed by ELISA.

Results: Proteomic analysis demonstrated that LPS induces the expression of numerous proteins involved in OA process in human OA synovial cells. In particular, it stimulates inflammation through the production of pro-inflammatory cytokines (Interleukin-6, IL-6), the catabolism through an increase of metalloproteases (MMP-1, MMP-3, MMP-13), and the production of pain-mediating neurotrophin (Nerve Growth Factor, NGF). These increases were observed at level of mRNA levels and of protein release. LPS also increases the amount of PGE2, another inflammation and pain mediator. At doses tested, vegetal extracts had little effects: only curcumin slightly counteracted the effects of LPS on NGF and MMP13 mRNA, and PGE2, IL-6 and MMP13 release. In contrast the association of curcumin with harpagophytum and bromelain reversed lots of effects of LPS in human OA synovial cells. It significantly reduced the gene expression and/or the release of proteins involved in catabolism (MMP3 and 13), inflammation (IL-6) and pain (PGE2 and NGF).

Conclusion: We show that the stimulation of human OA synovial cells with LPS permit to induce protein changes similar to an inflamed OA synovial tissues. In addition, using this model, we demonstrate that the combination of three vegetal compounds, namely curcumin, harpagophytum and bromelain have anti-inflammatory and anti-catabolic action in synovial cells and may thus reduce OA progression and related-pain.

Introduction

Osteoarthritis (OA) is a debilitating and painful disease characterized by inflammation of the synovial membrane and the progressive destruction of articular cartilage [1, 2]. It is one of the top ten causes of physical disability [3]; however, its etiology and pathogenesis are still not fully understood. Long

considered as a simple cartilage degenerative disease, OA is now described as a global joint chaos [4]. To date, no treatment has been able to reverse or alter OA progression.

Although OA is not classified as an inflammatory disease, many reports suggested that inflammation could be a major driver of OA development. Actually, elevated joint inflammation has been correlated with progression to the disease [5]. Thus, although OA pathogenesis remains unclear, inflammation is widely regarded as an extremely important factor for progression of this disease [2, 6–8].

Synovitis, i.e. inflammation of synovial tissues, is common in OA [9], and is mediated, in part, by fibroblast like synoviocytes (FLS). These cells play an important factors in OA inflammation and joint destruction, primarily by secreting a wide range of proinflammatory mediators, such as IL-6 and prostaglandin E2 (PGE2) [9], which leads the release of neurotrophins, such as NGF, participating to pain during OA, as well as the secretion of various type of proteases, including MMPs and a disintegrin and MMP with thrombospondin motifs (ADAMTS) [10], promoting the degradation of extracellular cell matrix (ECM), and further aggravating the progression of OA. Therefore, alleviating synovial inflammation may prevent the onset or minimize the progression of OA and symptoms [2, 11–13]. Conventional anti-inflammatory drugs are nonsteroidal anti-inflammatory drugs (NSAIDs) [14], however, they have several side effects and drug interactions including the risk of gastrointestinal, cardiovascular, and kidney problems. Used natural compounds may be a relevant alternative.

Herbal medicine has been used from ancient times to the present day for healing purposes. Curcumin (CUR) which is extracted from the rhizome of *Curcuma longa* L. is one of the most ancient medicinal herbs and is broadly used in human health due to its various therapeutic effects such as anti-inflammatory, antioxidant, anticancer and antimicrobial [15]. In patients with OA, oral administration of curcumin ameliorates the clinical manifestations of the disease [16–19], improves quality of life and enables a decrease in the consumption of NSAIDs [20]. This beneficial effect of curcumin is associated to its ability to reduce OA inflammation in cells, animal models, and human studies [21, 22]. The action of curcumin may be reinforced by combination with other natural compounds [15, 23].

The purpose of the present study was to investigate the effects of the combination of curcumin (CUR), bromelain (BRO), a food obtained from pineapple and having analgesic properties [24], and harpagophytum (HAR), a traditional remedy for articular diseases [25]), on inflammation in an original experimental *in vitro* model of osteoarthritis, using human synovial cells treated with lipopolysaccharide (LPS).

Material And Methods

Reagents

Lipopolysaccharide (LPS) from *E.Coli*, Sigma Aldrich, Saint Louis, USA) was dissolved in phosphate buffer saline without Calcium and Magnesium (DPBS, Lonza, Bâle, Suisse), to reach a concentration of 1 mg/ml and used at a final concentration of 1 µg/ml. Curcumin (Tumeric extract granule, 95%

curcuminoids, Natural, St Sylvain d'Anjou, France) was resuspended in dimethylsulfoxide (DMSO, Dutscher, Bernolsheim, Belgium). Bromelain (Bromelain 2500 GDU, Cambridge Commodities Ltd, Ely, UK) and Harpagophytum (*Harpagophytum procumbens*, Biosearch Life, Granada, Spain) extracts, the suspension was carried out in DPBS.

Culture cells and treatments

Human synoviocytes were recovered from the hip synovial membrane of 6 patients undergoing hip replacement surgery (age mean = 75 years). The cells were released by digestion of the synovial membrane with type I collagenase (2 mg/ml, 12 hours; ThermoFisher, Waltham, USA). The cells were incubated in Dulbecco's modified Eagle medium high glucose with glutamine and sodium pyruvate (DMEM, Dutscher), supplemented with 10% Fetal Bovine Serum (FBS, Dutscher), penicillin-streptomycin (Lonza) then incubated at 37 ° C in a humid atmosphere, containing 5% CO₂.

To achieve the desired number of cells, passages were performed. The cells were rinsed with DPBS, then detached with 0.05% trypsin (ThermoFisher). The cells were recovered in culture medium and seeded at approximately 7500 cells / cm². The absence of mycoplasmas were checked by PCR.

The cells were processed at confluence. Treatments were diluted in new culture medium to the desired concentration. Each molecule was tested alone or in the presence of LPS. The three molecules were also tested together in order to see the effects of the combination of these three extracts, in the presence or not of LPS.

Protein extraction

Cells were lysed and protein extracted using Radio Immuno Precipitation Assay (RIPA) Buffer (50mM Tris-HCl pH 7.5 ; 1% Igepal CA-630 ; 150mM NaCl ; 1mM EGTA ; 1mM NaF ; 0.25% Na-deoxycholate ; Distilled water) supplemented with protease inhibitor (Leupeptine 1mg / ml ; Phenyl methyl sulfonyl fluoride 200mM ; pepstatin A 1mg/ml) and phosphatase inhibitor (sodium orthovanadate 200mM) as previously described [26].

Proteomic experiment

Five µg of each protein extract were prepared using a modified Gel-aided Sample Preparation protocol [27]. Samples were digested with trypsin/Lys-C overnight at 37°C. For nano-LC fragmentation, protein or peptide samples were first desalted and concentrated onto a µC18 Omix (Agilent) before analysis.

The chromatography step was performed on a NanoElute (Bruker Daltonics) ultra-high-pressure nano flow chromatography system. Approximately 200ng of each peptide sample were concentrated onto a C18 pepmap 100 (5mm x 300µm i.d.) precolumn (Thermo Scientific) and separated at 50°C onto a reversed phase Reprosil column (25cm x 75µm i.d.) packed with 1.6µm C18 coated porous silica beads (Ionopticks). Mobile phases consisted of 0.1% formic acid, 99.9% water (v/v) (A) and 0.1% formic acid in 99.9% ACN (v/v) (B). The nanoflow rate was set at 400 nl/min, and the gradient profile was as follows:

from 2 to 15% B within 60 min, followed by an increase to 25% B within 30 min and further to 37% within 10 min, followed by a washing step at 95% B and reequilibration.

MS experiments were carried out on an TIMS-TOF pro mass spectrometer (Bruker Daltonics) with a modified nano electrospray ion source (CaptiveSpray, Bruker Daltonics). The system was calibrated each week and mass precision was better than 1 ppm. A 1600 spray voltage with a capillary temperature of 180°C was typically employed for ionizing. MS spectra were acquired in the positive mode in the mass range from 100 to 1700 m/z. In the experiments described here, the mass spectrometer was operated in PASEF mode with exclusion of single charged peptides. A number of 10 PASEF MS/MS scans was performed during 1.25 seconds from charge range 2–5.

Before post-process, the samples are analysed using Preview software (ProteinMetrics) in order to estimate the quality of the tryptic digestion and predict the post-translational modifications present. The result, below, is used for the “bank research / identification” part. The fragmentation pattern was used to determine the sequence of the peptide. Database searching was performed using the Peaks X + software. A UniProt *Homo sapiens* database (October 2020) was used. The variable modifications allowed were as follows: Nterm-acetylation, methionine oxidation, Deamidation (NQ), Methylation (KR) and Carbamylation. In addition, C-Propionoamide was set as fix modification. “Trypsin” was selected as Specific. Mass accuracy was set to 30 ppm and 0.05 Da for MS and MS/MS mode, respectively. Data were filtering according to a FDR of 0.5% and the elimination of protein redundancy on the basis of proteins being evidenced by the same set or a subset of peptides.

Identification of differentially expressed proteins

To quantify the relative levels of protein abundance between different groups, samples were analysed using the label-free quantification feature of PEAKS X + software. Feature detection was separately performed on each sample by the expectation-maximization based algorithm. The features of the same peptide from all replicates of each sample were aligned through the retention time alignment algorithms. Mass error tolerance was set at 30 ppm, Ion Mobility Tolerance (1/k0) at 0.07 and retention time tolerance at 10 min. Normalization factors of the samples were obtained by the total ion current (TIC) of each sample. Quantification of the protein abundance level was calculated using the sum area of the top three unique peptides. A 1.5-fold increase in relative abundance and a significance ≥ 5 using ANOVA as significance method were used to determine those enriched proteins

Enrichment analysis et comparison with datasets related to skeletal biology

Heatmap was performed with a Spearman clustering method using ComplexHeatmap package from R.

Enrichments in molecular process, cellular process and pathways (KEGG and Reactome) were performed using ClueGo App from Cytoscape software. Network specificity was set to medium; GO tree interval was set between 2 and 4. Clusters were performed using a selection set to 3 min genes in addition to 4% of genes. Enrichments were performed using a Bonferroni step down method.

Additionally, differentially expressed proteins were compared to existing gene expression datasets related to skeletal biology using SkeletalVis application (<http://skeletalvis.ncl.ac.uk/skeletal/>, [28]). Proteins encoded by genes associated with osteoarthritis joint damage in animals were also identified using OATargets databases [29].

RNA extraction and RT-PCR

RNA was extracted from the cell layer using the kit RNeasy mini kit (Qiagen, Hilden, Germany) according to the supplier's protocol. DNase treatment and the reverse transcription were, then, carried out using the kit DNase I (Sigma Aldrich) and the reverse transcriptase M-MLV (Invitrogen, Carlsbad, USA) as previously described [30]. Next, cDNA was amplified by real-time PCR using PCR master Mix (Power Syber Green, Applied biosystems, Courtaboeuf, France) and read on Step One Plus Real Time PCR system (Applied Biosystems) with the following primers: RPL13A Forward: 5'-GAGGTATGCTGCCCCACAAA-3' and Reversed: 5'-GTGGGATGCCGTCAAACAC-3'; NGF Forward: 5'-AGCGCAGCGAGTTTTGG-3' and Reversed: 5'-AGAAAGCTGCTCCCTTGGTA-3'; IL-6 Forward: 5'-CACACAGACAGCCACTCACC-3' and Reversed : 5'-TTTCACCAGGCAAGTCTCCT-3'; MMP1 Forward: 5'-GAAGCTGCTTACGAATTTGCCG-3' and Reversed: 5'-CCAAAGGAGCTGTAGATGTCCT-3'; MMP3 Forward: 5'-TAAAGACAGGCACTT TTGGCGC-3' and Reversed: 5'-TTGGGTATCCAGCTCGTACCTC-3'; MMP13 Forward: 5'-AAGGAGCATGGCGACTTCT-3' and Reversed: 5'-TGGCCCAGGAGGAAAAGC-3'. The relative mRNA level was calculated with the $2^{-\Delta\Delta CT}$ method. RPL13a was used as the invariant housekeeping gene internal control. The choice of this gene is based on our previously experience on the field.

ELISA

PGE2 and MMP release into conditioned media was quantified using commercially available enzyme immunoassay kit (R&D Biosystem) as previously [26]. For IL-6, we proceeded in the same way but using the Human beta-NGF ELISA Kit and Human IL-6 ELISA kit (Sigma Aldrich). The immunoassays were all carried out following manufacturer protocol. Absorbance was determined at 450 nm with a wavelength correction set at 540 nm using Multiskan GO spectrophotometer (Thermo Scientific).

Statistical analyses

All results are expressed as the mean of 3 or 4 patients (biological replicats) $\pm$ standard error of the mean (SEM). Statistical analyzes are carried out on GraphPad prism 8 software. After checking the normal distribution of samples, two-way ANOVA's tests were used for multiple comparisons. In significant cases, Tukey's multiple comparisons test for matched samples were performed as post-hoc analysis. P-values < 0.05 were considered significant.

Results

Stimulation of human OA synovial cells with LPS, an efficient OA model in vitro.

Lipopolysaccharide (LPS) is recently considered as a trigger for the pathology of OA, and is used to model inflammatory component of OA. So, we planned to test the effects of curcumin, harpagophytum and bromelain in human OA synovial cells stimulated with LPS. Before, we wanted to validate the model and its ability to model OA inflammation. So, we did proteomic analysis to define differential expressed proteins between unstimulated OA synovial cells and LPS-stimulated OA synovial cells. 2917 proteins were identified in the control group, and 3011 in the LPS treated-group. Among them, 106 proteins were differentially expressed between the two groups (Peaks Sign >5, Fold-change > 1.5, figure 1 and table 1). More precisely, 66 proteins (i.e. 62%) were significantly downregulated by LPS, and 40 (i.e. 38%) were upregulated by LPS. ClueGo analysis revealed that these differentially expressed proteins are mainly involved in the biological processes of oxidative stress-induced cell death (45%, p-value < 0.01) and in the molecular processes of intramolecular oxidoreductase activity (25%, p-value < 0.01) and collagen binding (12.5%, p-value < 0.01) (figures 2, tables 2 and 3). Furthermore, pathway enrichment by KEGG (figure 3A, table 4) showed the presence of proteins involved in protein digestion and absorption, fructose and mannose metabolism and antigen processing and presentation (33% for each, p-value < 0.01). Enrichment using Reactome (figure 3B, table 5) shows also the presence of proteins involved in assembly of collagen fibrils and other multimeric structures (24%, p-value < 0.05).

The comparison by signature of the proteomic profile between control and LPS stimulated synovial cells using skeletalvis database, which permits to explore skeletal biology related expression datasets [28], suggests that deregulated proteins were encoded by genes which are also differentially expressed in several other OA models (suppl data 1), namely "Synovial cells from inflammatory and normal areas of osteoarthritis synovial membrane" (signed jaccard (sig) = 0.015; zscore = 5.08) and observed in "Rat model of surgically induced knee osteoarthritis" (signed jaccard (sig) = 0.0118; zscore = 3.98). Besides, using OATargets databases [29], we could observe that several identified proteins were encoded by genes associated to OA, such as Thrombpondin-1 (THBS1), collagen alpha-1(VI) chain (COL6A1), superoxide dismutase [Mn] mitochondrial (SOD2) and Nicotinamide phosphoribyltransferase (NAMPT) (table 1). In addition, about half of these gene was also found at least once as a human OA DEG, and around 90% are known to interact with OA genes (table 1).

Together this proteomic analysis clearly confirms that LPS-stimulated synovial cells from OA human patients are good model to study osteoarthritis process *in vitro*.

LPS increases the expression of genes associated to inflammation, catabolism and pain

Next, using a most targeted strategy, we investigated the effect of LPS treatment in human OA synovial cells. After 24 hours of treatment, LPS stimulated inflammation through the production of pro-inflammatory cytokines (Interleukin-6, IL-6), the catabolism through an increase of metalloproteases (MMP-1, MMP-3, MMP-13), and the production of pain-mediating neurotrophin (Nerve Growth Factor, NGF). These increases were observed at level of mRNA levels and of protein release. LPS also increased the amount of PGE2, another pain mediator (figure 4).

The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to catabolism

Having validated our model, we continued by studying the effect of vegetal extracts (curcumin bromelain, and harpagophytum) on OA associated genes. At doses tested, vegetal extracts had little effects on the expression of catabolic genes. Only curcumin slightly counteracted the effects of LPS on MMP13 mRNA and protein release. However, the association of curcumin with harpagophytum and bromelain reversed effects of LPS on the mRNA levels of MMP1, MMP3 and MMP13, and on the release of MMP3 and MMP13 proteins (figure 5). These data suggest that the combination of curcumin, bromelain and harpagophytum may reduce cartilage degradation during OA process.

The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to inflammation and pain

Next, we investigated the effect of these vegetal compounds on the expression of genes involved in inflammation and pain (figure 6). We observed that only curcumin was able to slightly reduce the LPS-induced expression of NGF and the release of PGE2 and IL-6. Interestingly, the association of the three vegetal compounds (curcumin, harpagophytum and bromelain) significantly reduced the gene expression of IL-6 and NGF mRNA expression. It also decreases the IL-6 release and the production of PGE2. This suggest that the combination of the three compound may reduce inflammation and pain.

Discussion

To date, no efficient treatment exists to treat osteoarthritis. Consequently, the identification of strategies able to slow down OA progression and usable in the long term is crucial. Some natural compounds are known to present anti-oxidative and anti-inflammatory actions. So, they may be an alternative to pharmacological drugs. Herein, after a proteomic characterization of the *in vitro* OA model which was used in the study and validation that it is able to induce changes in gene expression profile similar to that is observed during OA, we showed that the combination of curcumin, harpagophytum and bromelain is efficient to counteract numerous LPS-induced effects in human OA synovial cells.

First, we evaluate the potential of lipopolysaccharide to induce changes in gene/protein expression mimicking some features of OA. LPS is an endotoxin and a classical activator of the innate immune system. Because of its pathophysiological properties, LPS has been used to induce arthritis in conjunction with collagen in animal models [31, 32]. More recently, researchers have started to connect LPS with the pathogenesis of OA [33]. LPS is released by gut microbiota and is correlated with the pathophysiology of osteoarthritis, in part through the activation of macrophages. In addition, local LPS administration to joints induce synovitis and is used as a model to evaluate potential treatments for acute synovitis [34].

Since LPS is now considered as a trigger for the pathology of OA, especially by activating synovial cells, we have proposed that stimulated human OA synovial cells may induce inflammation and reproduce *in*

vitro some changes observed during OA process. Using proteomics, we demonstrate here that treatment of human OA synovial cells with LPS induces the expression of signature genes of OA, and in particular reproduces some gene expression changes observed in synovial cells from inflammatory and normal areas of osteoarthritis synovial membrane. A more targeted strategy shows for instance that LPS induces the expression of MMPs, IL-6, PGE2 and NGF, which are mainly markers of catabolism, inflammation and pain in joints. Consequently, the stimulation of human OA synovial cells by LPS appears a good *in vitro* model to study inflammation during OA. Knowing that alleviating inflammation may prevent the onset or minimize the progression of OA [2, 11, 12, 33], we propose to use this *in vitro* model to test the ability of several natural substances to reduce inflammation.

First, we show that curcumin have some anti-catabolic and anti-inflammatory action in human OA synovial cells. This is in agreement with literature which demonstrate that curcumin reduces MMP-3 and MMP-13 expression in rabbit chondrocytes and in the articular cartilage of estrogen-deficient rats, preventing collagen degradation [35, 36]. Also, curcumin prevents the activation of nuclear factor kappaB (NF-κB), the major mediator of inflammation [36, 37]. Another study shows that curcumin favors cartilage anabolism by increasing type II collagen synthesis [22, 38].

We also investigated the effect of *Harpagophytum*, commonly known as devil's claw, a plant used worldwide as a traditional remedy for joint pain associated with OA and mild rheumatic ailments [25, 39, 40]. Moreover, it has been described to have analgesic effects on neuropathic pain in rats [41]. We also studied the effect of bromelain, a food supplement that is sometimes describes as an alternative treatment to nonsteroidal anti-inflammatory drug (NSAIDs) [42]. Bromelain has analgesic properties [43, 44] and relieves OA [24]. However, at dose tested, neither Harpogophytum nor bromelain show significant effect on the expression of studied genes, including NGF or PGE2, which are known to be related to joint pain. However, the association of these vegetal components with curcumin permits to counteract numerous effect of LPS in human OA stimulated cells. The combination of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to inflammation and pain, but also catabolism. This reinforced action of curcumin by combination with other natural compounds was already described [15]. For instance, the combination treatment with *Lactobacillus acidophilus* LA-1, vitamin B, and curcumin ameliorates the progression of osteoarthritis by inhibiting the pro-inflammatory mediators [23]. However, at our knowledge, this paper is the first to show the benefice to associated curcumin with bromelain and Harpagophytum.

In conclusion, we describe the changes in protein expression induced by LPS in human OA synovial cells and demonstrating that they are characteristic to inflamed OA synoviocytes, suggesting that this *in vitro* model may be useful to evaluate inflammation during OA. In addition, we showing that the combination of three natural vegetal components reduced expression of genes involved in catabolism, inflammation and pain, suggesting that together, they may present a beneficial effect on OA patients by alleviating OA pain and synovial inflammation, and reducing cartilage degradation.

Declarations

Ethics approval and consent to participate

The experimental protocol was approved by the local ethical committee “Comité de Protection des Personnes Nord-Ouest III” (agreement # A13-D46-VOL.19). The consent of each participant was obtained prior to surgery. They all signed agreement forms, in accordance with local law. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional file.

Competing interests

Thierry Conrozier received fees from LABRHA for scientific consultant and speaker services.

The other authors declare that they have no competing interests.

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This study was founded from Labhra laboratory, which supplied also vegetal compounds. The funding lab had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

CB participated in the conception, design of the study, analysis of data and drafted the manuscript. SB carried out the experiments and analysis data. BB and JP did proteomic experiments and analysis. TC participated to the coordination and conception of the study and design. KB participated in the conception and design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: List of deregulated proteins in LPS-stimulated synovial cells

Accession	Group Profile (Ratio)	Gene names (primary)	Description	OA associated	human OA DEG	induced OA DEG	OA gene interaction	skeletal phenotype
<i>Proteins down-regulated by LPS</i>								
Q92598	0.23	HSPH1	Heat shock protein 105 kDa	false		2	8	14 false
P49327	0.25	FASN	Fatty acid synthase	false		3	7	17 false
Q9Y3C0	0.26	WASHC3	WASH complex subunit 3	false		1	1	3 false
Q9H4B7	0.32	TUBB1	Tubulin beta-1 chain	false		5	1	3 false
P45877	0.36	PPIC	Peptidyl-prolyl cis-trans isomerase C	false		8	9	0 false
Q12768	0.43	WASHC5	WASH complex subunit 5	false		0	0	2 false
P07996	0.45	THBS1	Thrombopondin-1	true		4	2	19 true
Q8WWI1	0.45	LMO7	LIM domain only protein 7	false		3	5	7 false
Q8WX93	0.47	PALLD	Palladin	false		3	2	5 false
Q8NHP8	0.48	PLBD2	Putative phpholipase B-like 2	false		0	0	2 false
Q8NE86	0.49	MCU	Calcium uniporter protein mitochondrial	false		0	0	0 false
O60831	0.51	PRAF2	PRA1 family protein 2	false		1	3	0 false
Q9UMX0	0.52	UBQLN1	Ubiquilin-1	false		1	1	15 false
Q9NR12	0.52	PDLIM7	PDZ and LIM domain protein 7	false		5	3	7 false
O00154	0.52	ACOT7	Cytolic acyl coenzyme A thioester hydrolase	false		0	3	1 false
Q9Y305	0.53	ACOT9	Acyl-coenzyme A thioesterase 9 mitochondrial	false		0	4	6 false
Q71U36	0.53	TUBA1A	Tubulin alpha-1A chain	false		1	1	31 false
P15374	0.53	UCHL3	Ubiquitin carboxyl-terminal hydrolase isozyme L3	false		0	7	5 false
Q04760	0.53	GLO1	Lactoylglutathione lyase	false		0	3	3 true
P30419	0.53	NMT1	Glycylpeptide N-tetradecanoyltransferase 1	false		1	1	4 false
P55809	0.54	OXCT1	Succinyl-CoA:3-ketoacid coenzyme A transferase 1 mitochondrial	false		2	4	3 false
O43504	0.55	LAMTOR5	Ragulator complex protein LAMTOR5	false		0	1	4 false
P62841	0.55	RPS15	40S ribosomal protein S15	false		1	5	9 false
P36776	0.55	LONP1	Lon protease homolog mitochondrial	false		4	3	7 false
Q12849	0.55	GRSF1	G-rich sequence factor 1	false		0	1	2 false
Q5JRX3	0.55	PITRM1	Presequence protease mitochondrial	false		2	3	2 false
Q8TDQ7	0.55	GNPDA2	Glucamine-6-phosphate isomerase 2	false		0	1	1 false
P34932	0.56	HSPA4	Heat shock 70 kDa protein 4	false		0	2	39 false
Q15691	0.56	MAPRE1	Microtubule-associated protein RP/EB family member 1	false		0	0	13 false
P24539	0.56	ATP5PB	ATP synthase F(0) complex subunit B1 mitochondrial	false		0	0	6 false
P00491	0.56	PNP	Purine nucleide phosphorylase	false		6	3	1 false

P69905	0.56	HBA1; HBA2	Hemoglobin subunit alpha	false	7	0	3	false
Q15008	0.57	PSMD6	26S proteasome non-ATPase regulatory subunit 6	false	0	5	4	false
P02768	0.57	ALB	Albumin	false	2	0	9	false
Q9UHB6	0.57	LIMA1	LIM domain and actin-binding protein 1	false	2	4	9	true
Q15843	0.57	NEDD8	NEDD8	false	0	3	9	false
P15848	0.58	ARSB	Arylsulfatase B	false	3	6	1	true
O95202	0.58	LETM1	Mitochondrial proton/calcium exchanger protein	false	0	2	3	false
P12109	0.59	COL6A1	Collagen alpha-1(VI) chain	true	6	12	9	false
Q9UNZ2	0.59	NSFL1C	NSFL1 cofactor p47	false	0	1	8	false
Q9Y5L4	0.59	TIMM13	Mitochondrial import inner membrane translocase subunit Tim13	false	0	3	3	false
P55795	0.61	HNRNPH2	Heterogeneous nuclear ribonucleoprotein H2	false	0	2	5	true
Q9H008	0.61	LHPP	Phpholysine phphohistidine inorganic pyrophosphate phphatase	false	4	5	0	false
P12111	0.62	COL6A3	Collagen alpha-3(VI) chain	false	6	16	2	true
O14745	0.62	SLC9A3R1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	false	1	4	11	true
P98179	0.62	RBM3	RNA-binding protein 3	false	1	1	5	false
Q14318	0.62	FKBP8	Peptidyl-prolyl cis-trans isomerase FKBP8	false	1	2	10	true
O94826	0.62	TOMM70	Mitochondrial import receptor subunit TOM70	false	0	2	4	false
P48509	0.62	CD151	CD151 antigen	false	0	1	0	false
O75348	0.62	ATP6V1G1	V-type proton ATPase subunit G 1	false	1	2	0	false
P60953	0.63	CDC42	Cell division control protein 42 homolog	true	0	0	19	true
Q9BRA2	0.63	TXNDC17	Thioredoxin domain-containing protein 17	false	1	1	0	false
Q9BRF8	0.63	CPPED1	Serine/threonine-protein phphatase CPPED1	false	2	0	0	false
Q07666	0.64	KHDRBS1	KH domain-containing RNA-binding signal transduction-associated protein 1	false	0	2	16	true
Q16795	0.64	NDUFA9	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9 mitochondrial	false	0	2	6	false
O75431	0.64	MTX2	Metaxin-2	false	0	1	0	false
Q9C0H2	0.64	TTYH3	Protein tweety homolog 3	false	1	4	0	false
Q5JPE7	0.64	NOMO2	Nodal modulator 2	false	0	0	1	false
P69849	0.64	NOMO3	Nodal modulator 3	false	0	0	1	false
P00966	0.65	ASS1	Argininuccinate synthase	false	4	2	4	false
O75531	0.65	BANF1	Barrier-to-autointegration	false	0	2	2	false

			factor					
O95372	0.65	LYPLA2	Acyl-protein thioesterase 2	false	0	0	1	false
Q9P1F3	0.65	ABRACL	Ctars family protein ABRACL	false	3	2	0	false
Q15233	0.66	NONO	Non-POU domain-containing octamer-binding protein	false	0	2	16	false
P14324	0.66	FDPS	Farnesyl pyrophosphate synthase	false	0	2	3	false
P60983	0.66	GMFB	Glia maturation factor beta	false	1	2	1	false
<i>Protein up-regulated by LPS</i>								
Q96AT9	51.49	RPE	Ribulose-phosphate 3-epimerase	false	0	0	3	false
O14684	4.09	PTGES	Prostaglandin E synthase	false	8	4	0	false
P43490	3.35	NAMPT	Nicotinamide phphoribyltransferase	true	8	0	4	false
O43776	2.66	NARS1	Asparagine--tRNA ligase cytoplasmic	false	0	0	3	false
Q5VYK3	2.56	ECPAS	Proteasome adapter and scaffold protein ECM29	false	1	0	8	false
P12955	2.47	PEPD	Xaa-Pro dipeptidase	false	0	5	5	true
P10301	2.33	RRAS	Ras-related protein R-Ras	false	3	1	5	false
P19827	2.27	ITIH1	Inter-alpha-trypsin inhibitor heavy chain H1	false	0	1	1	false
P15121	2.04	AKR1B1	Aldo-keto reductase family 1 member B1	false	0	4	3	false
O14828	1.96	SCAMP3	Secretory carrier-associated membrane protein 3	false	1	1	4	false
P07711	1.95	CTSL	Cathepsin L1	false	4	0	3	false
Q6IBS0	1.92	TWF2	Twinfilin-2	false	0	5	0	false
P04179	1.9	SOD2	Superoxide dismutase [Mn] mitochondrial	true	7	7	8	false
P54709	1.85	ATP1B3	Sodium/potassium-transporting ATPase subunit beta-3	false	1	3	3	false
Q9Y3Z3	1.84	SAMHD1	Deoxynucleide triphphate triphphohydrolase SAMHD1	false	1	2	3	false
Q9Y3A6	1.84	TMED5	Transmembrane emp24 domain-containing protein 5	false	3	3	0	false
Q13501	1.79	SQSTM1	Sequestome-1	false	3	1	42	true
Q96JJ7	1.79	TMX3	Protein disulfide-isomerase TMX3	false	0	0	0	false
O75828	1.78	CBR3	Carbonyl reductase [NADPH] 3	false	2	5	3	false
P35613	1.78	BSG	Basigin	false	2	0	7	false
P26599	1.76	PTBP1	Polypyrimidine tract-binding protein 1	false	2	0	11	false
Q9Y295	1.76	DRG1	Developmentally-regulated GTP-binding protein 1	false	0	1	2	false
Q7L523	1.74	RRAGA	Ras-related GTP-binding protein A	false	0	3	2	false
P61009	1.7	SPCS3	Signal peptidase complex subunit 3	false	2	1	1	false
Q96HE7	1.69	ERO1A	ERO1-like protein alpha	false	5	1	1	false

Q9UL46	1.66	PSME2	Proteasome activator complex subunit 2	false	0	0	2	false
Q13724	1.63	MOGS	Mannyl-oligaccharide glucidase	false	0	2	5	false
Q9Y5P6	1.63	GMPPB	Manne-1-phphate guanyltransferase beta	false	2	2	1	false
P19525	1.61	EIF2AK2	Interferon-induced double-stranded RNA-activated protein kinase	false	1	1	18	false
P02794	1.59	FTH1	Ferritin heavy chain	false	3	1	5	false
P18085	1.58	ARF4	ADP-ribylation factor 4	false	2	10	10	false
P23381	1.57	WARS1	Tryptophan--tRNA ligase cytoplasmic	false	0	0	3	false
P63244	1.56	RACK1	Receptor of activated protein C kinase 1	false	1	0	22	false
Q9NZ08	1.56	ERAP1	Endoplasmic reticulum aminopeptidase 1	false	1	1	3	false
O95747	1.55	OXSRI	Serine/threonine-protein kinase R1	false	1	1	6	false
P17858	1.54	PFKL	ATP-dependent 6-phphofructokinase liver type	false	1	3	6	false
P08195	1.53	SLC3A2	4F2 cell-surface antigen heavy chain	false	6	0	7	false
Q9UNN8	1.53	PROCR	Endothelial protein C receptor	false	4	8	0	false
P04439	1.51	HLA-A	HLA class I histocompatibility antigen A alpha chain	false	1	0	5	false
P50991	1.5	CCT4	T-complex protein 1 subunit delta	false	0	3	11	false

Table 2 : Enrichment in biological processes

GOID	GOTerm	Term PValue	% Associated Genes	Nr. Genes	Associated Genes Found
GO:0006521	regulation of cellular amino acid metabolic process	0.007	4.48	3	[BSG, PSMD6, PSME2]
GO:0016667	oxidoreductase activity, acting on a sulfur group of donors	0.005	5.00	3	[ERO1A, TMX3, TXNDC17]
GO:0071230	cellular response to amino acid stimulus	0.001	5.56	4	[ASS1, COL6A1, LAMTOR5, RRAGA]
GO:0072523	purine-containing compound catabolic process	0.004	5.36	3	[ACOT7, PNP, SAMHD1]
GO:1901569	fatty acid derivative catabolic process	0.000	18.75	3	[ACOT7, LYPLA2, OXCT1]
GO:1901661	quinone metabolic process	0.001	8.11	3	[AKR1B1, CBR3, NDUFA9]
GO:1990928	response to amino acid starvation	0.003	5.88	3	[EIF2AK2, FASN, RRAGA]
GO:0034198	cellular response to amino acid starvation	0.003	6.25	3	[EIF2AK2, FASN, RRAGA]
GO:0070671	response to interleukin-12	0.005	5.08	3	[CDC42, PSME2, SOD2]
GO:0035722	interleukin-12-mediated signaling pathway	0.004	5.36	3	[CDC42, PSME2, SOD2]
GO:0040019	positive regulation of embryonic development	0.002	6.98	3	[AKR1B1, OXSR1, RACK1]
GO:0071470	cellular response to osmotic stress	0.004	5.66	3	[AKR1B1, LETM1, OXSR1]
GO:0051181	cofactor transport	0.005	5.17	3	[BSG, OXSR1, SLC9A3R1]
GO:0072337	modified amino acid transport	0.001	10.00	3	[BSG, OXSR1, SLC9A3R1]
GO:0061245	establishment or maintenance of bipolar cell polarity	0.004	5.66	3	[ARF4, CDC42, SLC9A3R1]
GO:0035088	establishment or maintenance of apical/basal cell polarity	0.004	5.66	3	[ARF4, CDC42, SLC9A3R1]
GO:0045197	establishment or maintenance of epithelial cell apical/basal polarity	0.003	6.25	3	[ARF4, CDC42, SLC9A3R1]
GO:0007006	mitochondrial membrane organization	0.000	4.05	6	[ATP5PB, HSPA4, LETM1, MTX2, NMT1, TIMM13]
GO:0051205	protein insertion into membrane	0.007	4.48	3	[HSPA4, NMT1, TIMM13]
GO:0090151	establishment of protein localization to mitochondrial membrane	0.003	5.88	3	[HSPA4, NMT1, TIMM13]
GO:0051204	protein insertion into mitochondrial membrane	0.003	6.38	3	[HSPA4, NMT1, TIMM13]
GO:1902882	regulation of response to oxidative stress	0.000	4.90	5	[BSG, NONO, RACK1, SOD2, UBQLN1]
GO:1902883	negative regulation of response to oxidative stress	0.000	6.67	4	[BSG, NONO, RACK1, SOD2]
GO:0036473	cell death in response to oxidative stress	0.000	5.00	5	[BSG, NONO, RACK1, SOD2, UBQLN1]
GO:1900407	regulation of cellular response to oxidative stress	0.000	5.38	5	[BSG, NONO, RACK1, SOD2, UBQLN1]
GO:1900408	negative regulation of cellular response to oxidative stress	0.000	6.90	4	[BSG, NONO, RACK1, SOD2]
GO:0008631	intrinsic apoptotic signaling pathway in response to oxidative stress	0.003	6.25	3	[NONO, SOD2, UBQLN1]
GO:1903201	regulation of oxidative stress-induced cell death	0.000	6.33	5	[BSG, NONO, RACK1, SOD2, UBQLN1]
GO:0036475	neuron death in response to oxidative stress	0.001	9.09	3	[BSG, NONO, RACK1]

GO:1903202	negative regulation of oxidative stress-induced cell death	0.000	6.90	4	[BSG, NONO, RACK1, SOD2]
GO:1903203	regulation of oxidative stress-induced neuron death	0.001	10.00	3	[BSG, NONO, RACK1]
GO:1902175	regulation of oxidative stress-induced intrinsic apoptotic signaling pathway	0.001	9.68	3	[NONO, SOD2, UBQLN1]
GO:1903204	negative regulation of oxidative stress-induced neuron death	0.000	13.64	3	[BSG, NONO, RACK1]

Table 3 : Enrichment in molecular functions

GOID	GOTerm	Term PValue	% Associated Genes	Nr. Genes	Associated Genes Found
GO:0005518	collagen binding	0.009	4.05	3	[COL6A1, CTSL, THBS1]
GO:0016790	thiolester hydrolase activity	0.000	10.00	4	[ACOT7, ACOT9, FASN, LYPLA2]
GO:0019210	kinase inhibitor activity	0.008	4.29	3	[GMFB, RACK1, WARS1]
GO:0042169	SH2 domain binding	0.002	6.52	3	[KHDRBS1, RACK1, SQSTM1]
GO:0042805	actinin binding	0.002	6.98	3	[LMO7, PALLD, PDLIM7]
GO:0051117	ATPase binding	0.002	4.30	4	[AKR1B1, ATP1B3, ATP6V1G1, NSFL1C]
GO:0016667	oxidoreductase activity, acting on a sulfur group of donors	0.005	5.00	3	[ERO1A, TMX3, TXNDC17]
GO:0016860	intramolecular oxidoreductase activity	0.000	6.67	4	[ERO1A, GNPDA2, PTGES, TMX3]

Table 4 : Enrichment using KEGG

GOID	GOTerm	Term PValue	% Associated Genes	Nr. Genes	Associated Genes Found
KEGG:00051	Fructose and mannose metabolism	0.002	9.09	3	[AKR1B1, GMPPB, PFKL]
KEGG:04612	Antigen processing and presentation	0.003	5.13	4	[CTSL, HLA-A, HSPA4, PSME2]
KEGG:04974	Protein digestion and absorption	0.005	4.21	4	[ATP1B3, COL6A1, COL6A3, SLC3A2]

Table 5 : Enrichment using reactome

GOID	GOTerm	Term PValue	% Associated Genes	Nr. Genes	Associated Genes Found
R- HSA:1268020	Mitochondrial protein import	0.001	6.25	4	[MTX2, PITRM1, TIMM13, TOMM70]
R- HSA:917937	Iron uptake and transport	0.009	5.17	3	[ATP6V1G1, FTH1, NEDD8]
R- HSA:9639288	Amino acids regulate mTORC1	0.008	5.45	3	[ATP6V1G1, LAMTOR5, RRAGA]
R- HSA:210991	Basigin interactions	0.001	12.00	3	[ATP1B3, BSG, SLC3A2]
R- HSA:2173782	Binding and Uptake of Ligands by Scavenger Receptors	0.000	9.52	4	[ALB, FTH1, HBA1, HSPH1]
R- HSA:447115	Interleukin-12 family signaling	0.009	5.26	3	[CDC42, PSME2, SOD2]
R- HSA:8950505	Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	0.003	7.89	3	[CDC42, PSME2, SOD2]
R- HSA:9020591	Interleukin-12 signaling	0.005	6.38	3	[CDC42, PSME2, SOD2]
R- HSA:1442490	Collagen degradation	0.012	4.69	3	[COL6A1, COL6A3, CTSL]
R- HSA:1474290	Collagen formation	0.005	4.44	4	[CD151, COL6A1, COL6A3, CTSL]
R- HSA:186797	Signaling by PDGF	0.009	5.17	3	[COL6A1, COL6A3, THBS1]
R- HSA:2022090	Assembly of collagen fibrils and other multimeric structures	0.001	6.56	4	[CD151, COL6A1, COL6A3, CTSL]
R- HSA:216083	Integrin cell surface interactions	0.004	4.71	4	[BSG, COL6A1, COL6A3, THBS1]
R- HSA:1632852	Macroautophagy	0.001	4.41	6	[LAMTOR5, RRAGA, SQSTM1, TOMM70, TUBA1A, TUBB1]
R- HSA:2995410	Nuclear Envelope (NE) Reassembly	0.019	4.00	3	[BANF1, TUBA1A, TUBB1]
R- HSA:389957	Prefoldin mediated transfer of substrate to CCT/TriC	0.001	10.71	3	[CCT4, TUBA1A, TUBB1]
R- HSA:389958	Cooperation of Prefoldin and TriC/CCT in actin and tubulin folding	0.002	9.38	3	[CCT4, TUBA1A, TUBB1]
R- HSA:389960	Formation of tubulin folding intermediates by CCT/TriC	0.001	12.00	3	[CCT4, TUBA1A, TUBB1]
R- HSA:5626467	RHO GTPases activate IQGAPs	0.002	9.38	3	[CDC42, TUBA1A, TUBB1]
R- HSA:8852276	The role of GTSE1 in G2/M progression after G2 checkpoint	0.000	6.49	5	[MAPRE1, PSMD6, PSME2, TUBA1A, TUBB1]
R- HSA:9663891	Selective autophagy	0.003	4.94	4	[SQSTM1, TOMM70, TUBA1A, TUBB1]

Figures

Figure 1

Figure 1

Heatmap showing differentially expressed proteins by LPS in human synovial cells Human synovial cells from three different patients were treated with LPS (1µg/ml) for 48h. At the end of experiments, proteins were extracted and proteomics analysis performed. Differentially expressed proteins between control group and LPS group is shown (n=3)

Figure 2

Figure 2

Enrichment in biological process and molecular function. From differentially expressed proteins between group control and LPS (figure 1), enrichments in biological process (A) and molecular process (B) were performed. Diagrams shows part of each GO Terms which were statistically enriched. *: p-value<0.05, **: p-value<0.01.

Figure 3

Figure 3

Enrichment in functional pathways. From differentially expressed proteins between group control and LPS (figure 1), enrichments pathways using KEGG (A) or Reactome (B) datasets were performed. Diagrams shows part of each GO Terms which were statistically enriched. *: p-value<0.05, **: p-value<0.01.

Figure 4

Figure 4

LPS induces gene expression and release in medium of markers of catabolism, inflammation and pain. Human synovial cells were treated with LPS (1µg/ml) for 24h. At the end of experiments, RNA was extracted. Relative mRNA expression of MMP-1, MMP-3, MMP-13, NGF and IL-6 was determined by RT-PCR. Values are compared to untreated cells and presented as log Fold Change (compared to control group). Culture medium were collected and ELISA performed to assayed MMP, IL-6 and PGE2

concentration in medium. Values are expressed as $\mu\text{g/ml}$ medium (n=4). *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

Figure 5

Figure 5

The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to catabolism Human synovial cells were treated with LPS (1 $\mu\text{g/ml}$) for 24h in the presence of of curcumin (13 μM) with bromelain (14.7 $\mu\text{g/ml}$), and harpagophytum (36 $\mu\text{g/ml}$),

or altogether. At the end of experiments, RNA were extracted and medium collected. Relative mRNA expression of MMP1, MMP3, MMP13 were determined by RT-PCR. Culture media were also collected and ELISA performed to assayed MMP release in medium. Values were compared to LPS-treated cells and presented as relative expression (compared to LPS group). n=3. *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

Figure 6

The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to inflammation and pain Human synovial cells were treated with (1 µg/ml) for 24h in the presence of of curcumin (13 µM) with bromelain (14.7 µg/ml), and harpagophytum (36 µg/ml), or altogether. At the end of experiments, RNA were extracted and medium collected. Relative mRNA expression of NGF and IL-6 were determined by RT-PCR. Culture media were also collected and ELISA performed to assayed IL-6 and PGE2 release in medium. Values were compared to LPS-treated cells and presented as relative release (compared to LPS group). n=3. *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

Supplementary Files

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- [Suppldata1SkeletalVis.xls](#)