attenuates NF-κB signalling through at least inhibition of DNA binding in HACs with attenuation of expression of several NF-κB dependent genes. SFN abrogates cytokine-induced destruction of bovine nasal cartilage at the level of both proteoglycan and collagen breakdown (10μM compared to cytokines alone). It also decreases arthritis score in the DMM murine model of osteoarthritis (3μmol daily dose SFN in diet versus control chow).
Conclusions: SFN, at levels which can be obtained through a broccoli-rich diet, inhibits the expression of key metalloproteinases implicated in osteoarthritis independently of Nrf2 and blocks inflammation at the level of NF-κB to protect against cartilage destruction in vitro and in vivo. Ongoing studies in man will ascertain the potential of this compound in human osteoarthritis.

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IDENTIFYING DIET-DERIVED CHONDROPROTECTIVE COMPOUNDS IN OSTEOARTHRITIS

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Purpose: Current pharmacological intervention for osteoarthritis (OA) is focused on inflammation and pain relief rather than addressing the degradation of articular cartilage. Such treatments typically have a single mode of action in a multifactorial disease. Compounds present in the habitual diet are as an attractive alternative, since foods typically contain multiple bioactive compounds that can interact with multiple cellular pathways. The purpose of this study was to identify novel chondroprotective compounds from the habitual diet.
Methods: Since matrix metalloproteinase-13 (MMP-13) is considered a key collagen-degrading enzyme, inhibition of MMP13 expression, measured by qRT-PCR, was used as a surrogate marker of cartilage degradation. Ninety-six diet derived compounds were selected from a list of compounds based on (i) the edibility of the source; (ii) how commonly it was consumed; (iii) whether the compound had previously been studied in chondrocytes. Compounds (at 10 μM) were screened in triplicate against basal expression and inhibition of interleukin-1 (IL-1)-induced expression of MMP13 in SW1353 chondrosarcoma cells and the C28/I2 immortalised human chondrocyte cell line. The lead compounds from these screens were then assayed in three isolates of primary human articular chondrocytes for their impact on expression of MMP13, MMP1, ADAMTS4 and ADAMTS5. Compound toxicity was measured using lactate dehydrogenase release and FACS.
Results: All compounds tested were non-toxic at 10μM. Six compounds significantly reduced IL-1-induced MMP13 expression in SW1353 cells, whilst eleven compounds significantly reduced IL-1-induced MMP13 expression in C28/I2 cells ($p<0.05$ - $p<0.0001$). Of these compounds assayed in primary human articular chondrocytes, five compounds significantly inhibited both IL-1-induced MMP1 and MMP13 expression (apigenin, aloe emodin, emodin, luteolin and isoliquiritigenin). Apigenin significantly inhibited IL-1-induced ADAMTS5 and aloe emodin significantly inhibited IL-1-induced ADAMTS4 ($p<0.05$ - $p<0.01$). Apigenin, aloe emodin and isoliquiritigenin showed dose-dependency across the 2.5 - 40μM range.
Conclusions: This screen has identified a number of target compounds which have the potential to be chondroprotective. Apigenin is a flavone found in various plants including celery and swede; aloe emodin is a hydroxyanthraquinone found in aloe vera; isoliquiritigenin is a chalcone from licorice. These compounds will now be taken forwards for further analyses.

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THE IN VITRO EFFECTS OF PROCYANIDINS AND HYDROXYTYROSOL-CONTAINING GRAPE AND OLIVE EXTRACT MIX ON THE INFLAMMATION-ASSOCIATED OSTEOARTHRITIS PROCESSES

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Purpose: Osteoarthritis (OA) is a major health concern that affects a growing part of our aging population associated with strong socio-economic burdens. To date, there is no curative treatment for OA. Pharmaceutical drugs alleviate inflammation and pain but none succeeds to slow down, stop or reverse the progression of the cartilage degradation and other adverse tissue injuries related to the pathology. In the last decades, many studies tried to demonstrate the effectiveness of herbal medicines to treat OA without the side effects associated with pharmacological treatments. This growing interest has led to the emerging concept of nutraceuticals. Among these nutraceuticals, phenolic phytochemicals compounds like procyanidins and hydroxytyrosol are now well acknowledged for their potent antioxidant and anti-inflammatory effects. There is, however, no scientific evidence to show the efficacy and safety of these compounds for OA prevention. In this study, we examined the effects of a grape and olive extract mix (OPCO) containing a high concentration of procyanidins and hydroxytyrosol on the in vitro and ex vivo inflammation-associated OA events.
Methods: OPCO (Grapsud, France) was characterized for its procyanidins and hydroxytyrosol contents by vanillin method and HPLC. Human articular choncrocytes (HAC), rabbit articular chondrocytes (RAC) and cartilage explants were harvested from tibial plateau and femoral condyles of 7 weeks-old New Zealand white rabbits and human cadavers. Cell viability was evaluated with a Methyl tetrazolium salt (MTS) assay. To mimick the inflammatory conditions of OA, cells were treated with IL-1β (1ng/mL) for 24 and 48H and culture media were then collected for nitric oxide (NO) and prostaglandin E2 (PGE2) measurements. The NO production was investigated by the Griess method, and PGE2 production was determined by Enzyme-linked immunosorbent assay (ELISA). The nuclear translocation of NF-κB (subunit p65) in HAC treated with IL-1β in the presence of OPCO was investigated by immunofluorescence using a specific antibody.
Results: Our results showed that OPCO contained 30% and 6.4% of procyanidin and hydroxytyrosol, respectively. MTS assay indicated that OPCO did not affect HAC and RAC viability. Our results also showed that IL-1β treatment induced a 3.5, 8 and 9.5 fold increase in the NO production in RAC, HAC and human explants, respectively. In addition, IL-1β treatment triggered a 7-, 33- and 1300-fold increase in PGE2 production in RAC, HAC and human explants, respectively. Interestingly, a 24H pretreatment of RAC, HAC and human explants with OPCO induced a significant reduction in the IL-1β-induced production of NO by 32%, 54%, and 60%, respectively. The IL-1β-dependent synthesis of PGE2 in RAC, HAC and explants was also reduced by about 75%, 97%, and 97%, respectively. Finally, whereas IL-1β was found to induce the nuclear translocation of p65 NF-κB, OPCO was shown to inhibit the IL-1β-induced nuclear translocation of p65 NF-κB in HAC.
Conclusions: In this study we have showed that a grape and olive extract, containing high amount of procyanidin and hydroxytyrosol, may carry out potent anti-inflammatory activities through the inhibition of IL-1β-driven NO and PGE2 production. In addition, our results strongly suggest that the anti-inflammatory activity of OPCO is likely to be mediated at least through the inhibition of p65 NF-κB pathway. Further in vivo experiments in adapted animal models of OA are now under investigation to determine whether grape and olive extracts may be promising nutraceuticals for the prevention of inflammation-associated OA symptoms.

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BENEFICIAL EFFECT OF 3-HYDROXYTYROSOL ON CHONDROCYTES EXPOSED TO OXIDATIVE STRESS

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Purpose: A major opportunity is represented by the search for food-derived molecules able to interfere with the processes involved in the pathogenesis or progression of chronic degenerative- and age-related diseases, such as osteoarthritis (OA). Recent findings attributed a potential role to autophagy in the regulation of the cellular response to several stress stimuli. Although its role is context- and tissue-dependent and still unclear, autophagy has been observed to decrease during aging and several age-related diseases, including OA. Here we address the question whether 3-hydroxytyrosol (HT) pre-treatment of chondrocyte cultures is able to reduce cell death, terminal chondrocyte differentiation and matrix degradation induced by oxidative stress. This natural compound is one of the major polyphenols present in olive oil

and, in addition to its powerful antioxidant activity, it may exert modulatory effects on different signalling pathways.
Methods: The immortalized human cell line C-28/I2 and primary cultures of human chondrocytes (prepared from fragments of articular cartilage obtained from adult OA patients undergoing knee arthroplasty) were treated with HT, 30 minutes before exposition to hydrogen peroxide to induce oxidative stress and samples were collected at different times. We tested cell viability through reading on MUSE® Cell Analyzer. The expression of genes and proteins known or potentially implicated in OA was evaluated by Real-Time RT-PCR assay and by Western blot method, respectively. By 6 different algorithms (TargetScan, miRanda, PICTAR4, miRDB, miRWalk, RNA22) bioinformatic analysis selected possible candidate microRNAs that could interact with targets of interest. Subsequently we verified by Real Time RT-PCR assay possible variations in the expression of these microRNAs after treatments.
Results: We evaluated the gene expression of runt-related transcription factor-2 and matrix metalloproteinase-13, two molecular markers of OA, in human primary chondrocytes cultured in monolayer. HT prevents the increase induced by oxidative stress in the mRNA amount of both markers. Moreover this compound is able to reduce significantly C-28/I2 cells death. In order to investigate a possible involvement of autophagic process in this context, we evaluated the protein expression of LC3 II (active form associated to autophagosomes), after oxidative stress in presence or in absence of HT. We found that HT induces an increase of protein levels of this autophagic marker, suggesting a possible mechanism of cytoprotection. This effect of HT on autophagy may be promoted by modulating several effectors such as AMP-activated protein kinase (AMPK) and the NAD-dependent deacetylase Sirtuin-1 (SIRT-1). Indeed HT causes the increase of phospho-AMPK and SIRT-1 protein levels. At the same time, this compound is able to decrease the amount of microRNA-9, which can target SIRT-1 mRNA thus modulating its expression.
Conclusions: This study suggests new molecular mechanisms by which HT may prevent some aspects of OA pathogenesis and progression. Indeed the beneficial action of HT in this context seems to be related to the stimulation of autophagy. These data indicate that HT can be considered an attractive tool for the prevention of OA.This work was supported by FIRB (Ministero dell'istruzione, dell'Università e della Ricerca, Italy) grant RBAP10KCNS.

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EFFICACY AND SAFETY OF GINGER IN OSTEOARTHRITIS PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED PLACEBO-CONTROLLED TRIALS

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Purpose: To assess the clinical efficacy and safety of oral ginger for symptomatic treatment of osteoarthritis (OA).
Methods: A systematic literature search was undertaken in the bibliographic databases: MEDLINE via PubMed from 1950, EMBASE via OVID from 1980, CINAHL via EBSCO from 1981, Web of Science from 1900, and Scifinder from 1907, as well as The Cochrane Central Register of Controlled Trials, all up to August 2013. Inclusion criteria were: Randomized trials comparing any oral ginger preparation (consisting only of extracts of ginger species) with placebo, where the participants were aged 18 or over with OA in any joint. Major outcomes were reduction in pain and improvement in physical function. Harm was assessed as withdrawals due to adverse events. The efficacy effect size was estimated using Hedges' standardized mean difference (SMD) and safety using the risk ratio (RR). Standard random-effects meta-analysis was used by default (confirmed by a fixed-effects model), and inconsistency was evaluated by the I-squared index (I^2).
Results: Out of 113 retrieved references, 108 were discarded, leaving five placebo-controlled trials (757 patients, mainly with knee and/or hip OA) for the meta-analysis. The majority reported relevant randomization procedures and blinding but applied an inadequate intention-to-treat (ITT) approach. Pain reduction following ginger intake revealed a low degree of heterogeneity among trials ($I^2 = 27\%$), with a statistically significant SMD of -0.30 (95% CI: [-0.50, -0.09], $P = 0.005$) in favour of ginger. Ginger showed a statistically significantly larger improvement in physical function than placebo SMD = -0.22 (95% CI: [-0.39, -0.04]; $P = 0.01$; $I^2 = 0\%$). Based on data from three trials (328 patients), patients given ginger were more than twice as likely to discontinue treatment due to adverse events compared to placebo (RR = 2.33; 95% CI: [1.04, 5.22]; $P = 0.04$; $I^2 = 0\%$).
Conclusions: Ginger was found modestly efficacious in reducing pain and improve physical function in clinical trials of patients with mainly knee and/or hip OA. Ginger was also reasonably safe since reported adverse events were mild and reversible. We judged the evidence to be of moderate quality, based on the small number of participants and non-adequate ITT analysis (with the latter risk of bias item leading to downgrading from high to moderate confidence in the estimates).

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CHONDROPROTECTIVE ACTIVITY OF ACTI-JOINT®, A COMBINATION OF CHONDROITIN SULFATE, GLUCOSAMINE AND A NATURAL INGREDIENT RICH IN HYALURONIC ACID

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Purpose: Osteoarthritis (OA) is a significant worldwide health problem owing to the progressive and debilitating nature of the condition, which results in high morbidity and a marked decrease in the quality of life. OA involves the progressive degeneration of articular cartilage with a remodeling of subchondral bone and synovitis.
The objective is to evaluate the potential anti-arthritic activity of Acti-Joint®, a formulation that includes CS Bioactive® (Chondroitin Sulfate 100% purity ,CS), Glucosamine (GLU) and Hyal-Joint®, a natural ingredient rich in sodium hyaluronate in an in vitro chondrocyte model and in the collagen-induced arthritis (CIA) model in rats.
Methods: An in vitro study was performed using human osteoarthritic chondrocytes cultured in alginate (n= 3) in order to evaluate the potential chondroprotective activity of Acti-Joint®. Effects on degradation of the extracellular matrix were determined by measuring metalloproteinase-1 (MMP-1) and metalloproteinase-13 (MMP-13) activities by ELISA (R&D kits). The synthesis of sulfated glycosaminoglycans (GAGs) was measured in chondrocytes by determining the incorporation of [^{35}S]sulfate into cetylpyridiniumchloride-precipitable (CPC) GAGs by liquid scintillation analysis.
The anti-arthritic activity was investigated in the CIA model in rats. Female rats with developing type II collagen arthritis (n = 11) were treated orally once daily with distilled water (Vehicle group) or Acti-Joint® (160 mg/kg) starting 10 days prior to disease induction through the end of the study (day 35). Dexamethasone, the positive control group, was administered once daily starting at day 0. The arthritic clinical score for each animal was examined starting at day 7. A clinical score was given to each individual paw and was based on 0-4 scale. The sum of all paws was calculated for each animal. In addition, a cytokine analysis on the left hind joint was performed: IL-6 and TNFα gene expression.
Results: The in vitro assays showed that Acti-Joint® leads to a statistically significant reduction of MMP-1 and MMP-13 activities ($p<0.05$) and also a stimulation of the synthesis of GAGs compared to the Control. Acti-Joint® showed better efficacy than the standard combination GLU + CS, being CS of a lower quality and uncertain traceability. In the experimental conditions of the in vivo study, we found that this formulation reduced about 10% the clinical score after 3 weeks of the arthritis induction. This was accompanied by a significant reduction of the pro-inflammatory cytokines IL-6 (74%) and TNFα (70%) levels compared to Vehicle group.
Conclusions: The present study indicates that Acti-Joint® has chondroprotective and anti-inflammatory activities. Thus, this natural