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FLAVOCOXID ACTS VIA AN ANTI-PEROXIDASE ACTIVITY ON CYCLOOXYGENASE ENZYMES, 5-LIPOXYGENASE INHIBITION AND A STRONG ANTIOXIDANT ACTIVITY TO MANAGE OSTEOARTHRITIS

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Purpose: Flavocoxid, a USFDA-regulated, prescription medical food for the clinical dietary management of osteoarthritis (OA) under the supervision of a physician, shows equivalent efficacy to NSAIDs in clinical studies with fewer side effects. Its exact mechanism of action, however, is poorly understood. The aim of these studies is to characterize flavocoxid's peroxidase/cyclooxygenase inhibition of cyclooxygenase-1 (COX-1) and COX-2 as well as its anti-5-lipoxygenase (5-LOX) activity. In addition, flavocoxid's antioxidant capacity is determined along with its effect on inducible inflammatory gene expression.
Methods: Purified enzymes were used to assess flavocoxid's anti-peroxidase and anti-cyclooxygenase activity on COX-1 and COX-2 as well as inhibitory activity on 5-LOX. Multiple standard antioxidant assay assays were utilized to judge flavocoxid's antioxidant capacity and cell co-culture of LPS-stimulated human peripheral blood mononuclear cells (PBMCs) with flavocoxid for its effect on inducible inflammatory gene expression of COX-2, interleukin-1beta (IL-1β), IL-6 and tumor necrosis factor-alpha (TNFα) as well as nuclear factor-kappa B (NF-κB).
Results: Flavocoxid showed balanced inhibition of COX-1 and COX-2 peroxidase activities with inhibitory concentrations (IC_{50}s) of 12.3 and 11.3μg/ml, respectively, while the 5-LOX IC_{50} was 110μg/ml. No detectable 5-LOX inhibition was found for rofecoxib, celecoxib, valdecoxib, diclofenac, meloxicam, naproxen, ibuprofen or aspirin. Flavocoxid showed minimal inhibition of cyclooxygenase activity for COX-1 (IC_{50}=25μg/ml) compared to indomethacin (IC_{50}=0.012μg/ml) and no detectable cyclooxygenase inhibition of COX-2 compared to NS-398 (IC_{50}=0.095μg/ml). Flavocoxid also demonstrated a strong antioxidant capacity against a variety of reactive oxygen species: oxygen radical absorbance capacity, $ORAC_{hydro}$=3700 μmolTE/g; $ORAC_{lipo}$= 19μmolTE/g; ferric reducing/antioxidant power, FRAP=1145μmolTE/g; hydroxyl radical absorbance capacity, HORAC= 1326μmolCAE/g; peroxynitrite radical averting capacity, NORAC=1936μmolTE/g; superoxide radical averting capacity, SORAC= 27kunitSODeq/g; trolox equivalent antioxidant capacity, TEAC=2456μmolTE/g; and 2,2-di(4-*tert*-octylphenyl)-1-picrylhydroxyl capacity, DPPH=767μmolTE/g. In lipopolysaccharide-stimulated PBMCs, flavocoxid strongly reduced gene expression of COX-2 (80-fold), TNFα (11-fold), IL-1β (10-fold) and IL-6 (40-fold) and, to a lesser extent, COX-1 (2.8-fold). Expression of NF-κB gene was also reduced (2.2-fold).
Conclusions: These results suggest that the clinically favorable effects and equivalency to NSAIDs in the management of OA are more than likely achieved by simultaneous modification at multiple points in the inflammatory process making flavocoxid a unique anti-inflammatory acting *via* antioxidant mechanisms both on the cyclooxygenase enzyme and in damping inducible, inflammatory gene expression. Flavocoxid also acts through direct inhibition of 5-LOX to avoid a shunting of arachidonic acid metabolism toward leukotriene production.

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MEDICATION USE IN THE OSTEOARTHRITIS INITIATIVE (OAI) STUDY

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Purpose: The purpose of this study was to determine the frequency of prescription and the changes in prescribing patterns within the total cohort and three sub-cohorts of the OAI study.
Methods: Information was collected at baseline, 12, 24 and 36 month clinic visits and medication data were sorted by major drug classes, focussing on NSAIDs, Statins and Bisphosphonates, chosen as they are known to be associated with musculoskeletal diseases in each of the three cohorts (Progression, Incidence and Control). Chi-squared statistics were used to compare groups at baseline and 36 months.
Results: All cohorts contained more women than men and women were prescribed more items than men at all timepoints and in all cohorts except the control cohort at 36 months.
Figure 1 shows the major trends between cohorts and over time. Analysis

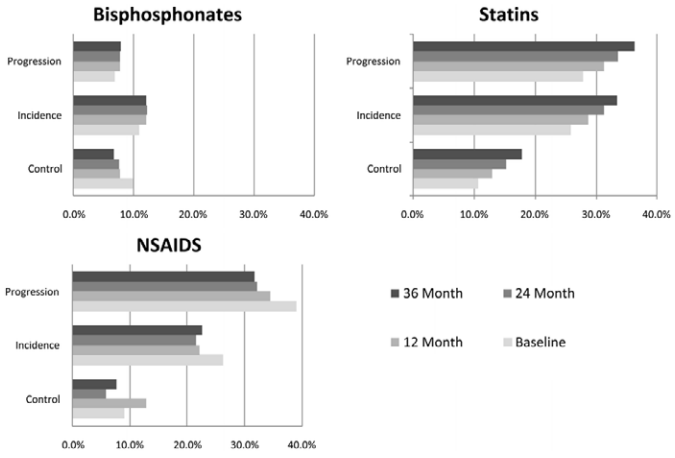

Figure 1

showed a significant increase in prescriptions of Statins from baseline to 3 years follow-up (from 26% to 36% of total cohort [n=4796 at baseline] ($P<0.0001$)). Atorvastatin prescription numbers did not change but there were increases in Simvastatin and Lovostatin prescription numbers in both the Progression and Incidence cohorts over time. There was a significant reduction in prescription of NSAIDs ($P<0.05$) although there was a 12.5% reduction in prescriptions of Alendronate.There was a decreasing trend for the prescription of anti-depressant drugs. This was noticeable in the prescriptions of Fluoxetine and Paroxetine which both decreased over time. Three subjects were noted to be on antiretroviral medication which, according to the FDA list of side effects, can result in severe bone loss. Opioid drug prescriptions did not change significantly over time.
The proportion of medications prescribed for each cohort varied significantly for each of the three drug groups ($P<0.001$). Fewer Bisphosphonates were prescribed in the Progression group (7%) compared to the Incidence (11%) or Control (10%) groups. Both Statin and NSAID prescriptions were highest in the Progression group (28% and 39% respectively), followed by the Incidence (26% and 26%) and Control groups (11% and 9%).
Conclusions: During the 3 years of this study, prescriptions of Statins significantly increased, NSAID prescriptions significantly decreased whilst Bisphosphonate prescriptions remained stable. The increase in prescriptions of Statins may reflect a general rise in the tendency to prescribe this medication in the 'normal' population. The reduction in COX-2 specific NSAIDS may be due to the withdrawal of Rofecoxib and limitations placed on the prescription of other COX-2 inhibitors.
There are drugs which we have considered under their major drug classification which may be prescribed for other purposes, such as Gabapentin which has been classified as treatment for epilepsy but can also be prescribed for severe neuropathic pain.
The changes in prescription use between the cohorts and over time should be borne in mind for all analysis. Bisphosphonate use is of particular interest, as it shows a large number of the cohort (over 450 subjects) are taking medication designed to increase bone density which may affect their OA status and progression in unknown ways.

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ANTI-INFLAMMATORY ACTIVITY OF AN ETHANOLIC *CAESALPINIA SAPPAN* EXTRACT IN HUMAN CHONDROCYTES *IN VITRO*

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Purpose: Extracts from *Caesalpinia sappan* as well as their main constituents have been reported to exhibit anti-inflammatory, antioxidant, antibacterial and immunosuppressive activities in different tissues. Given their inhibitory effect on inflammatory cytokines and mediators, components of *Caesalpinia sappan* might be potential drug candidates for disease modification in osteoarthritis (OA). The aim of this study was to evaluate the anti-inflammatory effects of an ethanolic extract from *Caesalpinia sappan* (CSE) in an osteoarthritic chondrocyte model.
Methods: CSE was prepared by continuous extraction with ethanol for 24 h. The quality and stability of CSE from different batches was verified