Flavocoxid (Limbrel®) manages osteoarthritis through modification of multiple inflammatory pathways: a review

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ABSTRACT:

Limbrel (flavocoxid) is marketed as an FDA-regulated medical food for the clinical dietary management of osteoarthritis (OA) to be used under physician supervision. Flavocoxid is composed of a >90% mixture of baicalin and catechin and represents a non-targeted anti-inflammatory which works differently than non-steroidal anti-inflammatory drugs (NSAIDs) that bind to and only inhibit the *cyclooxygenase* moieties of COX-1 and COX-2. Flavocoxid binds to and weakly modulates the *peroxidase* activity of the COX enzymes permitting low level expression of prostaglandins (PGs), prostacyclin (PGI_2) and thromboxane (TxA_2). In addition, flavocoxid weakly inhibits phospholipase A2 (PLA_2) and 5-lipoxygenase (5-LOX) as well as increases IκBα and prevents nuclear factor kappa B (NFκB) activation/induction of inflammatory genes such as tumor necrosis factor-alpha (TNFα), interleukin-1β (IL-1 β), IL-6, COX-2, inducible nitric oxide synthase (iNOS) and 5-LOX. In clinical studies, flavocoxid shows equivalent efficacy to naproxen with statistically fewer renal (edema) and upper gastrointestinal (GI) side effects, does not affect platelet function and bleeding times, does not change international normalized ratio (INR) in warfarinized patients, is well-tolerated in patients with previous NSAID-induced GI side effects, and decreases or eliminates the use of gastroprotective medications in patients who previously required them to tolerate NSAIDs. With its broad, non-targeted and multiple weak activities which result in fewer side effects compared to NSAIDs, flavocoxid represents a different way managing OA by working on the underlying and multiple causes of cartilage degradation as well as joint inflammation.

Keywords: Flavocoxid, Limbrel, osteoarthritis, inflammatory pathways, and medical foods

REVIEW:

Second only to beta-lactam drugs, NSAIDs, as a class, are the leading cause of acute drug reactions and drug-induced side effects [1]. Although multiple treatment modalities are used to

manage OA, including physical therapy, analgesics, intra-articular injections of corticosteroids or hyaluronate preparations and surgical interventions, NSAIDs remain the mainstay of most chemical therapeutic regimens. While effective for relieving pain, these drugs often lead to GI, renal, hepatic, blood clotting and cardiovascular toxicities. Most of these adverse effects are caused by imbalances in arachidonic acid (AA) metabolism due to specific inhibition of COX-1, COX-2 and 5-LOX changing the eicosanoid generation profile which serve important physiologic functions both within and beyond articular structures [2, 3]. Even with multiple NSAIDs and modalities for treatment of OA, this chronic and debilitating disease remains a serious problem affecting 25-40 million people in the United States [4-6] with similar data reported in other countries [7]. Epidemiological studies show that Asian and Mediterranean countries have a substantially lower incidence of knee OA, suggesting a role for nutrition in the etiology of the disease [8]. For example, Asian and Mediterranean countries consume an equal proportion of inflammatory omega-6 (precursors to AA production) to anti-inflammatory omega-3 fatty acids compared to Americans who consume about a 20-30:1 ratio of these molecules [9]. Another way to manage the effects of imbalances in nutrition which result in chronic diseases is through the administration of medical foods.

The Medical Foods Category. Medical foods, a therapeutic category unique to the US, are ideally positioned to provide the clinical dietary management of chronic diseases like OA. Unlike drugs, medical foods are not FDA approved, but are subject to a set of governing statutes and regulations specific to this class of therapeutics. In addition, despite a similar name to dietary supplements, medical foods have strict requirements for safety, labeling and use (see below). Medical foods are required to support all claims with good laboratory and clinical science. The category of medical foods was created in an addendum to the Orphan Drug Act of 1988 [10]. Prior to this law, these products were regulated as drugs. The definition of a medical food was restated in the FDA's Final Rule on Mandatory Nutritional Labeling, 1993 [11]. Specifically, a product classified as a medical food must meet the following requirements, among others:

- It is composed of specially formulated and processed nutrients (as opposed to naturally occurring foodstuffs used in their natural state or extractions of plants) at concentrations which cannot be attained by dietary modification. These nutrients must provide for a distinct nutritional requirement for disease management.

- It must be consumed or administered enterally, either by ingestion or intragastric tube.

- It provides nutritional support specifically modified for the management of the unique nutrient needs that are associated with the specific disease or condition, as determined by medical evaluation.

- It is intended to be used under medical supervision (i.e., by prescription, provided from a physician's office or in a clinical setting such as a hospital).

- It is intended only for a subject receiving active and ongoing medical supervision wherein the subject requires medical care on a recurring basis for a chronic condition(s) or disease(s).

- It is composed of ingredients designated as generally recognized as safe (GRAS). For an ingredient to achieve GRAS status requires not only a technical demonstration of non-toxicity,

but also an agreement of that safety after extensive peer review by an independent panel of experts in the field.

- It is based on recognized scientific principles (i.e., preclinical and clinical science).
- It must list its molecular ingredients in descending order on the label and provide clear directions to assure their safe use under physician supervision.

Based on this strict definition and regulatory structure, medical foods represent an option which physicians may choose for their patients to provide dietary management of a chronic condition or disease under physician supervision.

Distinct Nutritional Requirements in Osteoarthritis.

The prerequisite that there be a "distinct nutritional requirement" to manage OA with a medical food has evolved over many years as scientists and clinicians understand more about nutrition and joint damage. The role of nutrition in joint disease is complex and may be linked to nutritional deficiencies early as well as later in life [12]. The excess intake of inflammatory omega-6 fatty acids and the lack of natural anti-inflammatory compounds in the diet to mitigate this consumption in the US diet, for example, is part of the etiology of the growing incidence of OA. This is commensurate with a concept known as dietary-genetic discordance [13]. Briefly, this refers to the fact that our genetic makeup has not changed significantly in the 50,000 years, or so, since the days of early hominids. Genetically, our metabolic pathways are programmed to account for diets that consisted primarily of fruits, berries, vegetables, fish and lean meat which have high levels of anti-inflammatory and antioxidant molecules such as flavonoids and other polyphenols and an omega-6/omega-3 ratio of approximately 2:1. With the advent of agriculture and animal husbandry 10,000 years ago our diets started to become discordant with our metabolic genetic constitution and this discordance was further and dramatically altered with the advent of highly processed foods around the beginning of the twentieth century. The net result is that we now consume a diet low in natural antioxidants such as flavonoids and high in omega-6 fatty acids for which we do not possess appropriate metabolic pathways.

Arachidonic acid is derived from the dietary intake of omega-6 fatty acids (i.e., linoleic acid) as well as AA itself. Omega-6 fatty acids are processed by sequential desaturation and elongation [14]. Fatty acid imbalances are commonly seen in patients with chronic inflammatory conditions such as arthritis. Osteoarthritic joints show increased levels of lipid and AA accumulation which correlate with histological severity in joint disease [15, 16]. Total fatty acid levels in subchondral bone have also been shown to be 50%-90% higher in OA patients compared to controls or to osteoporotic individuals [17]. Similarly, bone marrow lesions in OA have been found to contain high levels of mono-, poly- and omega-6 polyunsaturated fatty acids compared to non-diseased control groups [18]. Dietary lipids have also been shown to modify the fatty acid composition of cartilage [19]. In addition, clinical studies have shown a strong linkage between metabolic defects in fatty acid metabolism or an overabundance of fatty acids that lead to OA [20]. A recent study of over 500 patients who had or were at high risk for developing OA showed a clear association between knee synovitis as assessed by MRI and omega-6 fatty acid intake [21]. It is unclear whether increased intake of omega-3 fatty acids can reverse the effect of AA in treating OA in humans. Clinical trials assessing the efficacy of omega-3 fatty acids in human OA to date have reached conflicting conclusions and formulations

tested thus far tend to contain mixtures of omega-3 fatty acids with other compounds [22, 23]. Therefore, it is not known if AA presence in OA joints can be decreased by dietary intervention with omega-3 administration. It is clear, however, that other dietary molecules which are lacking in the US diet can be supplied at high levels to manage the metabolism of AA overabundance associated with OA.

Dietary flavonoid intake has been shown to mitigate the occurrence and severity of many chronic diseases, especially those with inflammation as part of their etiology [24-26]. Americans and Northern Europeans consume fewer natural anti-inflammatory and antioxidant molecules compared to Asian and Mediterranean countries [24, 27-31]. These differences appear to be largely due to food processing differences which destroy flavonoid compounds and to food preferences [28, 32]. In addition, there is an inverse relationship between socioeconomic level and flavonoid as well as antioxidant intake in the US [33, 34].

Recent experiments in preclinical transgenic male mice which can express human C-reactive protein (CRP) show that a high-fat diet significantly induces higher OA grades in comparison to mice on normal chow [35]. There was no correlation between the severity of OA observed and body weight suggesting that a low-grade metabolically-based inflammatory state and not obesity or mechanical load leads to OA severity. In humans, flavonoid intake has also been shown to be inversely related to CRP levels suggestive of systemic inflammation in males and females of all ages [36-39]. Flavonoids have been shown to affect arthritis in both animals and humans.

Numerous models of experimental arthritis which induce high levels of reactive oxygen species (ROS) have shown that flavonoids from a variety of sources slow the progression and mitigate the effects of inflammation in rheumatoid arthritis (RA) [40-42]. Flavonoids like nobiletin from citrus, for example, decrease the production of aggrecanases which directly degrade cartilage in collagen-induced arthritis (CIA) models [43]. It has also been shown that initial joint injury which is associated with eventual joint degradation in OA is related to increases in ROS. When articular cartilage chondrocytes were taken from young adults and exposed to mechanical and oxidative stress, there was increased release of ROS and induction of chondrocyte senescence [44]. Animal models have also shown that compression injury increases ROS levels which induce apoptosis and maturation of chondrocytes [45]. Finally, in experimental models of articular cartilage stress, addition of antioxidants mitigated chondrocyte death [46, 47]. These observations suggest that joint injury increases ROS production, chondrocyte senescence and death and that these effects are mitigated by the addition of antioxidants. Similar findings have also been demonstrated in a number of human studies. It is clear that initial traumatic injury is associated with a change in joint tissue and an increased generation of ROS which eventually leads to OA.

Using food intake questionnaires, the Framingham Study demonstrated an association between vitamin C, beta carotene and vitamin E intake with the incidence and progression of OA compared to a panel of non-antioxidant vitamins [48]. High intakes of vitamin C were associated with a reduced risk of developing knee pain suggesting a decrease in the progression of OA [48]. Baker et al [49], in a longitudinal study of 324 participants followed for 30 months who were evaluated by food questionnaire, showed that individuals who consumed either 200 mg/day (men) or 150 mg/day (women) of vitamin C had significant reduction in knee pain. In addition,

293 healthy patients without knee pain or injury were assessed for intake of antioxidant vitamins and food sources including those containing carotenoids by a food frequency questionnaire at baseline and 10 years later and compared with measurements of bone area, cartilage defects and bone marrow lesions assessed by MRI [50]. High vitamin C intake was associated with a reduced risk of bone marrow lesions and reduced tibial plateau bone area. There was also an inverse association between fruit intake and bone area as well as bone marrow lesions. It was also found that intake of the carotenoids lutein and zeaxanthin reduced cartilage defects while β-cryptoxanthin was inversely associated with reduction of tibial plateau bone area. These results suggest the need for antioxidants to modulate joint damage. Indeed, multiple investigators suggest that OA can be managed by the addition of flavonoids and antioxidants to treatment regimens [51-54]. The accumulated evidence of all of these studies strongly supports the "distinct dietary requirement" of antioxidants in the form of vitamins and polyphenolic compounds such as flavonoids and carotenoids for the management of OA. With this in mind, flavocoxid was specially formulated to provide for these distinct dietary requirements.

**Characterization of Flavocoxid's Mechanism of Action:**
Baicalin and catechin, the constituents of flavocoxid, were isolated by high throughput cyclooxygenase (using COX-1 and COX-2 *peroxidase* inhibition screening) and 5-LOX inhibition screening of over 5000 plant extracts [55]. As a medical food, flavocoxid is required by FDA regulations to be produced under food good manufacturing practices (GMPs). Flavocoxid, however, is manufactured using pharmaceutical standards which involves setting specific ingredient ranges for each active entity, testing and setting stability for shelf-life of the product as well as testing for contaminating molecules such as pesticide [56] and liver damaging aflatoxins [57] which may be present due to growing conditions. This review represents a current summary of flavocoxid's mechanism of action, preclinical and clinical safety and efficacy data, and compares the clinical research of other nutritional molecules for the management of OA.

Flavocoxid Arachidonic Acid Mechanism. Arachidonic acid is derived from both PLA_2 conversion of cell membrane phospholipids and dietary consumption of omega-6 fatty acids [58]. This fatty acid is a necessary substrate for a variety of physiological processes, including those involving cell membrane composition, platelet function, inflammation and tissue function and repair. In OA and many other diseases, COX-1, COX-2 and 5-LOX enzymes produce effectors of pain and inflammation which are derived from AA. All three enzymes play a key role in the metabolism of AA to inflammatory fatty acids (eicosanoids) which contribute to the deterioration of cartilage (Figure 1).

During the metabolism of AA by the COX enzymes, two oxygen molecules are added by a *cyclooyxgenase* activity to AA to yield prostaglandin G2 (PGG_2) [59] (Figure 2). The transient, short-lived PGG_2 intermediate is then converted to prostaglandin H2 (PGH_2) by a *peroxidase* activity *via* a reduction reaction. A variety of cellular and tissue specific *isomerases* and *synthases* then convert PGH_2 to various PGs, PGI_2 and TxA_2 (Figure 2).

It was originally thought that COX-1 and COX-2 metabolic differences in AA conversion were due to compartmentalization of each enzyme on different structural components within the cell. Both enzymes, however, are equally present on the endoplasmic reticulum and nuclear

membranes [60]. The *cyclooxygenase* activity of COX-2 requires ~10-fold less hydroperoxide for activation compared to COX-1 and metabolizes AA at a greater rate [61]. Both COX-1 and COX-2 produce maintenance levels of the vasodilator PGI_2 from human endothelial cells [62].
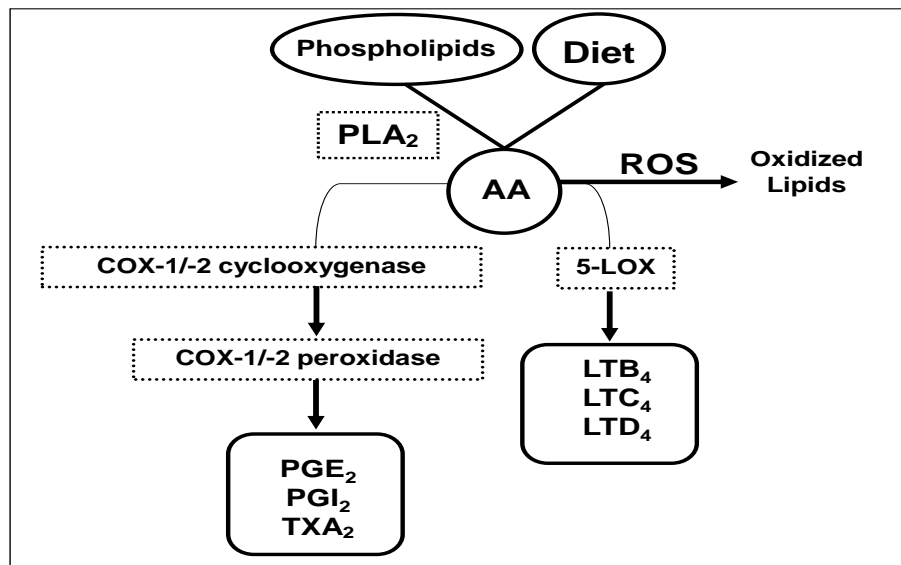
Figure 1. Pathways of origin and processing of arachidonic acid (AA) to eicosanoid fatty acid metabolites through the COX-1/-2 *cyclooxygenase* and *peroxidase* activities to prostaglandin E2 (PGE_2), prostacyclin (PGI_2) and thromboxane (TxA_2), through the 5-LOX enzyme to the leukotrienes LTB_4, LTC_4 and LTD_4 and the chemical change of AA to oxidized lipids by ROS.
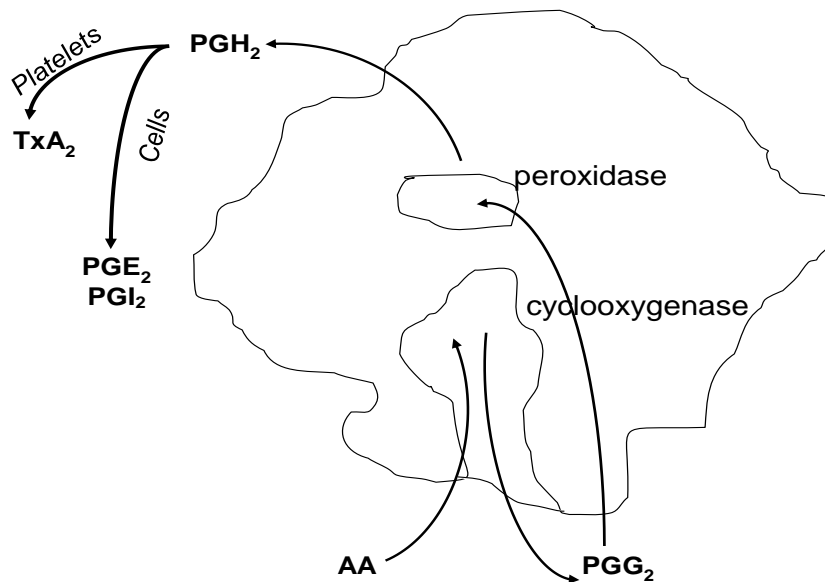
Figure 2. Processing of arachidonic acid (AA) by the COX enzyme activities of *cyclooxygenase* to prostaglandin G2 (PGG_2) and *peroxidase* to prostaglandin H2 (PGH_2) to eicosanoid metabolites of thromboxane (TxA_2) as well as prostaglandin E2 (PGE_2) and prostacyclin (PGI_2)

Prostacyclin is, however, produced at a faster rate by COX-2 compared to COX-1 [63]. In addition, each isozyme is coupled to specific *synthases* and/or *isomerases* for the final conversion of the PGH_2 intermediate from each COX enzyme in many cell types and platelets

[58]. For example, PGI_2 is specifically produced in the cardiovascular system through coupling of COX-2 with PGI_2 *synthase*, while TxA_2 production is coupled in platelets to COX-1 and TxA_2 *synthase*.

The initial testing of the mechanism of action (MOA) for flavocoxid focused on the effect of the baicalin and catechin mixture on inhibition of COX-1 and COX-2 *peroxidase* activities and the 5-LOX enzyme [64]. The initial analysis showed a balanced, weak inhibition of the *peroxidase* activities in the COX enzymes (15 µg/ml) and of 5-LOX (29 µg/ml). Flavocoxid also reduced PGE_2 in cultures of HOSC cells, a human osteosarcoma cell line expressing COX-2, and leukotriene (LTB_4) production in cultures of THP-1 cells, a monocyte cell line that expresses COX-1, COX-2, and 5-LOX.

In a recently published paper, oxygen sensing assays for the *cyclooxygenase* activity and *peroxidase* inhibition were used to separate the two activities of the COX enzymes comparing indomethacin (a strong COX-1 *cyclooxygenase* inhibitor) and NS-398 (a strong COX-2 *cyclooxygenase* inhibitor) to flavocoxid [65]. The assays revealed a relatively balanced anti-*peroxidase* activity of flavocoxid on COX-1 (IC50=12.3 µg/ml) and COX-2 (IC50= 11.3 µg/ml) and only a weak COX-1 *cyclooxygenase* activity (IC50=25 µg/ml). Flavocoxid gave no detectable COX-2 *cyclooxygenase* inhibition in this oxygen sensing assay. Indomethacin yielded a IC50 of 0.012 µg/ml, 2000-fold stronger than flavocoxid, and NS-398 gave an IC50 of 0.095 µg/ml in the oxygen sensing assay. Flavocoxid was the only inhibitor of 5-LOX enzyme (IC50=110 µg/ml) compared to rofecoxib, valdecoxib, celecoxib, meloxicam, diclofenac, ibuprofen, naproxen and aspirin, all of which showed no inhibitory activity.

Though NSAIDs bind only to the *cyclooxygenase* site in the COX enzymes preventing the conversion of AA to PGG_2 thus stopping the process of eicosanoid generation, it is hypothesized that redundant cellular *peroxidase* activities can convert PGG_2 to PGH_2 to eicosanoid products *via* alternate pathways in target organs. This hypothesis is supported in humans administered 500 mg flavocoxid BID for 2 weeks in a platelet function study in which TxA_2 production was unaffected [66]. Traditional NSAIDs are known to decrease the production of TxA_2 from the COX-1 enzyme [67]. The anti-5-LOX activity of flavocoxid may also prevent a putative shunt of AA metabolism toward leukotriene (LT) production which NSAIDs (including selective COX-2 inhibitors) have been shown to promote based on both preclinical and clinical results [68]. Finally, flavocoxid demonstrated a weak anti-PLA_2 activity (IC50=60 µg/ml) in macrophage cultures. By broadly modulating the production of AA from phospholipids and its metabolism by COX in a manner different from NSAIDs and preventing a 5-LOX shunt of AA metabolism to produce LTs which contribute to NSAID-induced side effects, flavocoxid may allow for preservation of eicosanoid production in extra-articular locations to minimize AEs of the gut, kidneys and cardiovascular system.

Flavocoxid Antioxidant Mechanism: Non-enzymatic lipid peroxidation is another important pathway of AA metabolism. Lipid peroxidation, as a result of poor oxidative status, destabilizes cell membranes leading to induction of calcium dependent PLA_2 which hydrolytically attacks phospholipids leading to the generation of AA [69]. In joints, breakdown of chondrocyte cell membranes provides the substrate for PLA_2 conversion to AA [70]. When AA is exposed to ROS, it is oxidized to F2-isoprostanes, 4-hydroxynonenal (HNE), and malondialdehyde (MDA)

[71]. These oxidative AA conversion products are elevated in the synovial fluid, synoviocytes and serum of OA patients when compared to healthy control subjects and have been shown to stimulate the production of cartilage-degrading matrix metalloproteinases [72, 73]. In addition to the chemical effects of ROS on AA oxidative products, ROS and cytokines induce transcriptional activation of NFκB (Figure 3).
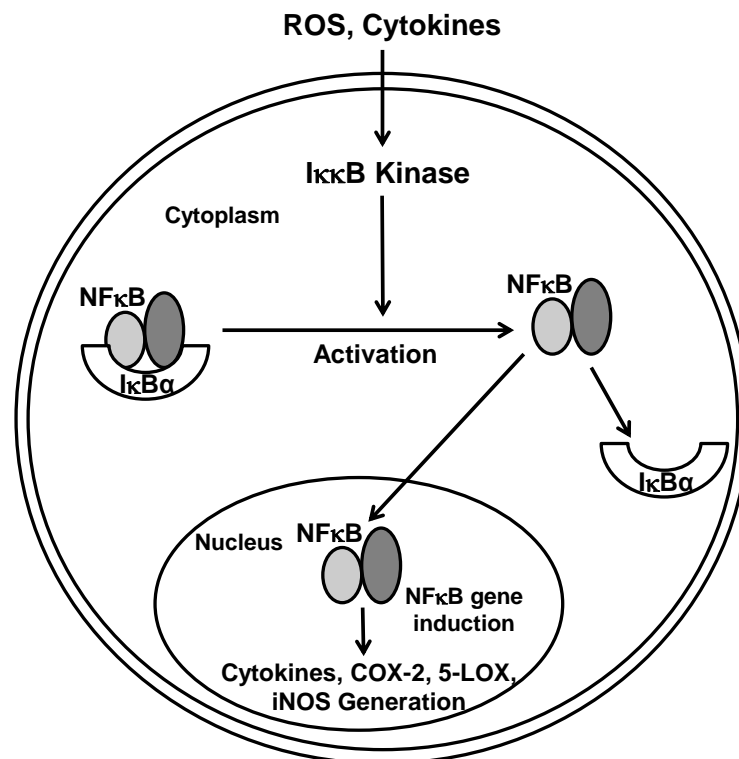

Figure 3. Reactive oxygen species (ROS) and cytokine activation of nuclear factor kappa B (NFκB) pathway of inflammatory gene induction.

Although NFκB is essential in normal physiology, inappropriate induction of NFκB is part of the pathogenesis of arthritis [74, 75]. Radical oxygen species play a major role in signaling the production of inflammatory gene expression by activating the nuclear transcription factor NFκB in joint tissue in both RA and OA [76-78]. Nitric oxide (NO) in particular induces proinflammatory cytokines (i.e., IL-1β, TNFα, IL-6) through NFκB activation [79, 80]. NFκB heterodimers are normally present in the cytoplasm bound by IκBα [81]. Reactive oxygen species and a variety of inflammatory signals bind to integral membrane receptors triggering IκκB kinase which phosphorylates IκBα causing it to dissociate from NFκB heterodimer (Figure 3). The controlling factor IκBα is fated for digestion by the proteosome. Activated NFκB translocates to the nucleus and binds to specific promoter sequences ahead of inflammatory genes called response elements recruiting coactivator proteins and RNA polymerase to transcribe the gene into mRNA. The mRNA coding sequence for the inflammatory gene is then translated into proteins, such as cytokines, COX-2, 5-LOX and iNOS [82, 83] (Figure 3). Flavonoid molecules have the ability to not only act as antioxidants, but also to directly down-regulate NFκB activity affecting inflammatory gene and protein expression in joints.

Using a variety of *in vitro* measures, flavocoxid was shown to have a broad and strong antioxidant profile. ORAC analysis provided a measure of the scavenging capacity of antioxidants against the peroxyl radical, which is one of the most common ROS found in the body [84]. The ferric reducing/antioxidant power (FRAP) assay was performed as previously described [85]. A validated assay for hydroxyl radical absorbance capacity (HORAC) was also performed according described methods [86]. The peroxynitrite radical averting capacity assay (NORAC) and superoxide radical averting capacity (SORAC) assays were performed as described previously [87]. Other antioxidant capacity assays used in the analysis of flavocoxid's antioxidant capacity include TEAC (trolox equivalent antioxidant capacity), a method developed by Rice-Evans' group and broadly applied in analyzing food samples [88] and DPPH [2,2-di(4-tert-octylphenyl)-1-picrylhydroxyl], an easy and accurate method frequently used to measure the antioxidant capacity of fruit and vegetable juices and extracts [89]. Based on these analyses, flavocoxid neutralized a broad spectrum of oxidative species such as hydroxyl radicals and ions, peroxynitrite, nitric oxide and peroxyl radicals (Table 1).

This broad antioxidant effect may help to explain the fact that flavocoxid significantly down-regulated COX-2 gene expression in isolated human peripheral blood monocytes from OA patients whereas celecoxib, ibuprofen, and to a minor extent, acetaminophen, augmented COX-2 gene expression (Figure 4) [From 65].
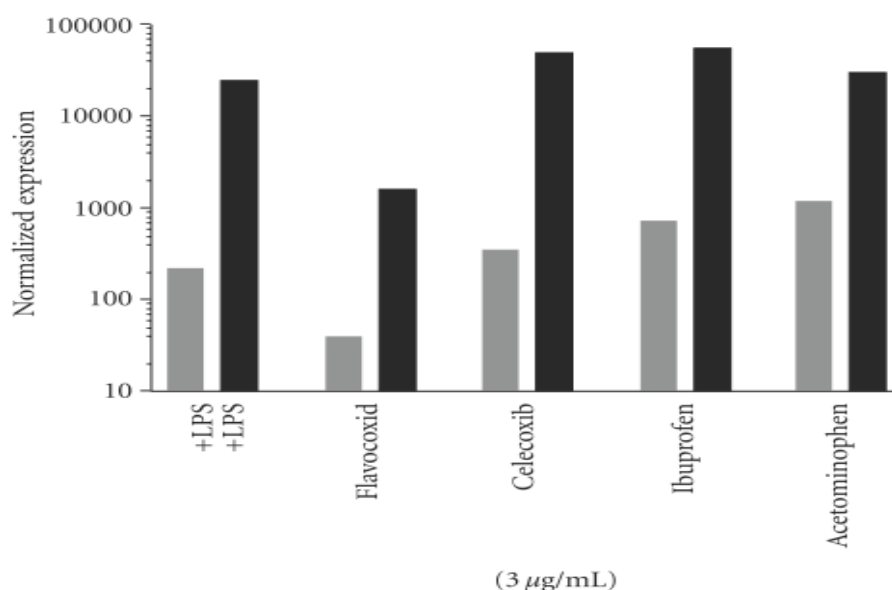
Figure 4. Effect of flavocoxid versus celecoxib, ibuprofen, and acetominophen on LPS-induced COX-1 (gray) and COX-2 (black) gene expression. Human peripheral blood mononuclear cells were isolated and co-cultured with lipopolysaccharide (LPS) at 10 ng/mL and 3 μg/mL in separate wells of flavocoxid, celecoxib, ibuprofen, or acetaminophen. Total RNAs were prepared and cDNAs synthesized. Quantitative PCR assays were run in duplicate primer and probe sets for COX-1 and COX-2 genes. Cyclophilin A was used as the reference transcript for the relative quantification of RNA levels to normalize gene expression [From 65].

This was a surprising result since both traditional and selective COX-2 NSAIDs bind to and inhibit the COX enzyme production of inflammatory AA metabolites. Similar results have been

found in patients who experience ulcerations in which COX-2 gene expression is up-regulated [90]. While this may be advantageous in maintaining prostaglandin E2 (PGE_2) production in the upper GI tract to mitigate ulcer formation, COX-2 gene induction could result in greater protein production generating elevated PG production associated with increased pain and inflammation. More complete gene expression analyses have also been performed with flavocoxid.

**Table 1.** Antioxidant profile of flavocoxid

	ORAC$_{hydro}$ (µmolTE/g)	ORAC$_{lipo}$ (µmolTE/g)	ORAC$_{total}$ (µmolTE/g)	HORAC (µmol CAE/g)	NORAC (µmol TE/g)	SORAC (kunit SODeq/g)	FRAP (µmol TE/g)	TEAC (µmol TE/g)	DPPH (µmol TE/g)
flavocoxid	3700	19	3719	1326	1936	27	1145	2456	767

ORAC$_{hydro}$=Oxygen Radical Absorbance Capacity reflects water-soluble antioxidant capacity; ORAC$_{lipo}$=Oxygen Radical Absorbance Capacity lipid-soluble antioxidant capacity; ORAC$_{total}$=Combined ORAC$_{hydro}$ and ORAC$_{lipo}$; HORAC=hydroxyl radical absorbance capacity; NORAC= peroxynitrite radical averting capacity; SORAC=superoxide radical averting capacity; FRAP=ferric reducing/antioxidant power; TEAC=trolox equivalent antioxidant capacity; DPPH=2,2-di(4-*tert*-octylphenyl)-1-picrylhydroxyl assay; TE=trolox equivalents; CAE=caffeic acid equivalents; SODeq=superoxide dismutase equivalents [From 65].

Quantitative PCR (qPCR), microarray analysis and ELISA were used to analyze flavocoxid's effect on inflammatory gene and protein expression [91, 92]. Whether analyzed by qPCR or DNA microarray, flavocoxid reduced lipopolysaccharide (LPS)-induced inflammatory gene expression including NFκB, IL-1β, TNFα, IL-6 and COX-2 while normalizing gene expression for a variety of genes in immortalized human monocytes as well as monocytes isolated from OA patients. The induced inflammatory protein levels, as assessed by ELISA, corresponded to the effects seen in gene expression assays. In another set of experiments, flavocoxid was shown to decrease malondialdehyde, a peroxyl radical product of AA oxidation, in macrophage cultures [93]. In the same study, NFκB activation and binding to a model promoter sequence was prevented and IκBα levels increased suggesting a restoration of NFκB regulation (Figure 5). This study also showed that flavocoxid-induced down-regulation of mRNA and protein levels of TNFα and iNOS as well as decreased protein levels of COX-2 and 5-LOX without effecting COX-1 protein expression. As a consequence of decreased COX-2, 5-LOX and iNOS proteins, there were decreased levels PGE_2, LTB_4 and NO (as the stable nitrite marker), respectively [93].

This MOA data was further demonstrated by Messina et al [94] in a Duchene muscular dystrophy (DMD) animal model. Part of the etiology of DMD is induction of inflammatory pathways through an oxidative mechanism. Currently, corticosteroids are the only treatment option for DMD patients. In this animal model, flavocoxid: 1) increased forelimb strength; 2) normalized strength to weight and decreased fatigue; 3) reduced serum creatine-kinase levels; 4) limited the protein expression of oxidative stress markers and of inflammatory mediators COX-2, 5-LOX and TNFα; 5) inhibited NFκB and mitogen-activated protein kinases (MAPKs) signal pathways p38 and JunK; 6) reduced muscle necrosis and enhanced regeneration equivalent to methylprednisolone. These results suggest that flavocoxid could be used as a therapy in DMD to possibly avoid the toxic, long-term side-effects of steroid treatment. A proof-of-principle safety

trial is currently underway in Duchene children [95]. Preclinical work has also been performed in other disease models on the effect of flavocoxid on oxidatively induced inflammation.
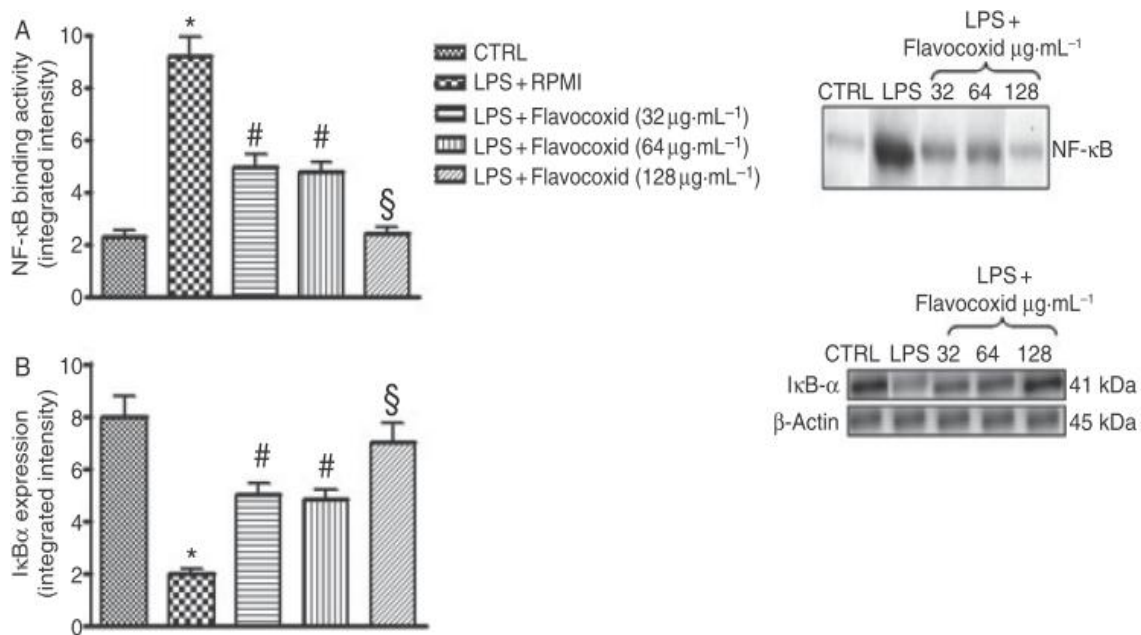
Figure 5. Electrophoretic mobility shift assay (EMSA) of NFκB binding activity in the nucleus (A) and Western blot analysis of IκBα proteins levels (B) in the cytoplasm of macrophages stimulated for 1 h with 1 µg/mL of LPS or its vehicle (1 mL of RPMI). Lipopolysaccharide (LPS)-stimulated macrophages were co-incubated with flavocoxid (32, 64 and 128 µg/mL) or RPMI alone. Bars represent the mean ±SD of seven experiments. *$P < 0.05$ versus control, [#]$P < 0.01$ versus LPS + RPMI, [##]$P < 0.005$ versus LPS + RPMI, [§]$P < 0.001$ versus LPS + RPMI [From 93].

In acute pancreatitis, inflammation triggers the expression of pancreatic enzymes which "autocatalytically" digest the pancreas [96]. This is followed by massive infiltration of leukocytes which causes local and systemic inflammatory responses often having fatal consequences. All of these reactions are triggered by ROS damage and induction of inflammatory pathways. In an experimental model of acute pancreatitis, Sprague-Dawley rats were administered flavocoxid 30 min after injection of caerulein [97]. Flavocoxid acted as a rescue therapy inhibiting COX-2 and TNFα gene expression as well as 5-LOX activation. In addition, flavocoxid reduced induced levels of PGE_2 and LTB_4, decreased serum lipase and amylase levels and protected against the histological damage in terms of cellular vacuolization and leukocyte infiltration.

Inflammation also plays an important role in the development of benign prostate hyperplasia (BPH). Products derived from the COX and 5-LOX are significantly elevated in the enlarging prostate [98, 99]. In rats injected subcutaneously daily with testosterone propionate (3 mg/kg) for 14 days to induce BPH, flavocoxid reduced prostate weight and hyperplasia, decreased the inducible expression of COX-2 and 5-LOX and, as a consequence, of limiting

PGE_2 and LTB_4 generation, enhanced pro-apoptotic Bax and caspase-9 levels and decreased anti-apoptotic Bcl-2 mRNA [100]. Flavocoxid also reduced epidermal growth factor and vascular endothelial growth factor expression. In human prostate carcinoma PC3 cells, flavocoxid-stimulated apoptosis and inhibited growth factor expression. These data suggest a role for flavocoxid-mediated apoptosis during prostatic growth.

Work has also been performed in a preclinical model of sepsis. In a cecal ligation and puncture (CLP) model performed in C57BL/6J mice, intraperitoneal injection of flavocoxid improved survival, reduced the protein expression of NFκB, COX-2, 5-LOX, TNFα and IL-6 and increased IL-10 production [101]. Moreover, flavocoxid inhibited the mitogen-activated protein kinases (MAPKs) pathway, preserved β-arrestin2 expression, significantly reduced blood LTB_4, PGE_2, TNFα and IL-6, and significantly increased anti-inflammatory IL-10 and lipoxin A4 serum levels compared to vehicle. Finally, flavocoxid protected against CLP-induced histological damage and reduced the myeloperoxidase activity in lung and liver tissue. These data suggest that flavocoxid protects mice from the inflammatory consequences of sepsis and may represent a promising option for therapy in the future.

The importance of this antioxidant mechanism cannot be underestimated, especially for the management of chronic discomfort which occurs in OA. All the molecules produced from the COX-2, 5-LOX and iNOS pathways (e.g., PGs, LTs, and NO) bind to and activate pain receptors to cause nociceptive signals that are transmitted to the brain. In addition, cytokines also bind these receptors as do a myriad of ROS. Flavocoxid functions to modulate all these pathways to affect inflammation and pain perception involved in OA. The overall cellular mechanism is represented in Figure 7A showing that flavocoxid acts broadly at many points in the inflammatory cycle as opposed to NSAIDs which principally act by decreasing eicosanoid production (Figure 7B).

Flavocoxid Preclinical Safety and Efficacy:
Flavocoxid was tested in cell and two rodent models for toxicity before being tested clinically. In human immortalized monocytes, flavocoxid yielded no cytotoxicity even at concentrations up to 100 µg/ml compared to aspirin and celecoxib which induced cell death at 50 and 33 µg/ml, respectively [102]. Acute and subchronic animal toxicology data in male and female ICR mice showed a lack of any harmful effects with human equivalent doses up to 10 g/day (acute) compared to placebo. There was also no gastric toxicity in Fischer 344 rats, a species sensitive to NSAIDs, as shown by histology of the stomach mucosa in this study. Flavocoxid also demonstrated no apparent drug interactions by standard CYP450 enzyme inhibition testing in human liver microsomes [102]. There was also no mutagenesis of DNA observed in AMES assays. This work in ICR mice and Fischer 344 rats was recently repeated in a more extensive animal toxicology study in Sprague-Dawley rats [103]. Yiman et al [103] demonstrated no flavocoxid-related mortality or ophthalmologic, neurologic (functional observational battery and motor activity), body weight, feed consumption, clinical observation, organ weight, gross finding, clinical or histopathological alterations at dose levels of 250, 500, and 1000 mg/kg/day (equivalent to 2822, 5645 and 11290 mg per day in a 70 kg human, doses far in excess of those given clinically). Normal sperm count and comparable estrus staging were also found for all animals tested. A dose of 1000 mg/kg/day was identified as the NOAEL (no-observed adverse-

effect-level) in this study. Studies were also performed in animal models to assess the effect of flavocoxid in inflammatory models.
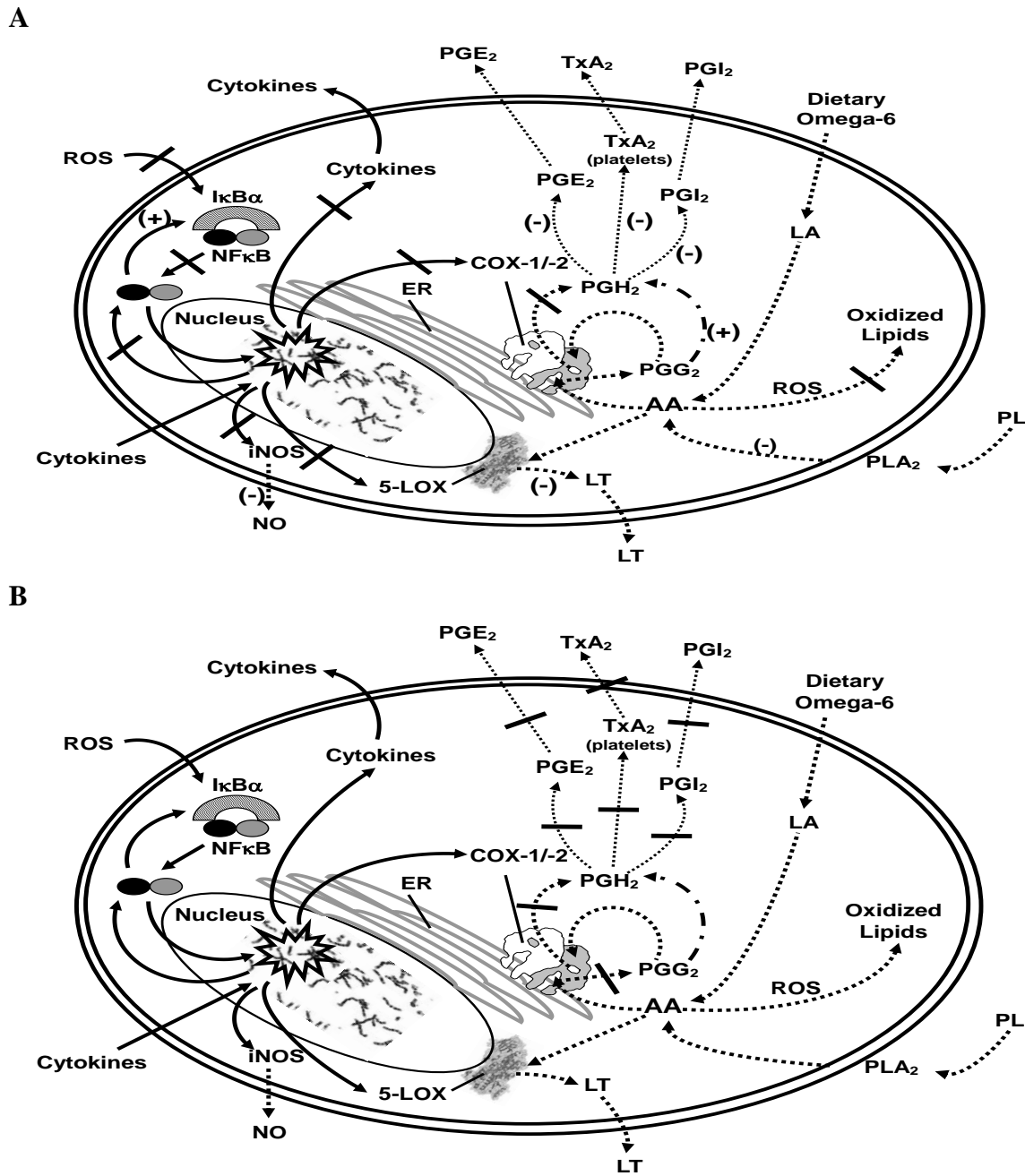
Figure 7. Broad mechanism of action of flavocoxid (A) versus NSAIDs (B). Flavocoxid acts to up-regulate (+) elements which damp NFκB activation thereby decreasing the expression of inducible inflammatory factors such as NFκB itself, cytokines, COX-2, 5-LOX and iNOS (/). Flavocoxid down-regulates or modulates (-) specific pathways of inflammation modifying the production and conversion of AA to eicosanoids and NO produced by inducible nitric oxide synthase (iNOS).   Prostaglandin H2 (PGH$_2$), the precursor to prostaglandin E2 (PGE$_2$), prostacylin (PGI$_2$) and thromboxane (TxA$_2$), is produced through an alternate pathway (+). NSAIDs work primarily at one point in the COX enzymes, the *cyclooxygenase* site, inhibiting

the production of prostaglandin G2 (PGG_2) which results in decreased PGH_2 generation (/) and a consequent reduction in PGE_2, PGI_2 and TxA_2 formation (/).

Reductions in ear and ankle swelling induced by AA in animal models were initially used to show flavocoxid's efficacy *in vivo* [64]. To determine if flavocoxid had an effect on inflammation *in vivo*, a validated mouse ear swelling assay with topically applied AA was utilized [104]. Twelve hours before applying AA to the back of one ear using the other as an internal control, ICR mice were gavaged with either flavocoxid or indomethacin. Microcaliper measurements demonstrated that flavocoxid showed an equivalent response in mitigating AA-induced ear swelling. After confirming the effects of flavocoxid on AA-induced ear swelling, AA was injected into the ankle joint of mice previously administered flavocoxid. Flavocoxid ameliorated ankle swelling compared to the non-treated animals. In addition, the decrease in swelling in ankle joints correlated with improved performance in a walking model on a rotating rod [64].

A head-to-head trial of a flavocoxid formulation for animals against a chondroitin sulfate/glucosamine hydrochloride/manganese ascorbate formulation (Cosequin©DS) in canines with OA [105] confirmed the superiority of flavocoxid with comparable safety. In this multi-site, double-blind, randomized, direct-comparator trial in dogs weighing at least 15 lbs (n=33), flavocoxid showed statistically significant improvement in pain scores, almost twice the rate of the chondroitin sulfate/glucosamine hydrochloride/manganese ascorbate (n=36) formulation using veterinarian and owner visual analog scale (VAS) assessments over a 2-month period. The incidence of AEs was low and generally mild in both groups [105].

Lastly, flavocoxid has been tested in a model of collagen induced arthritis (CIA) for RA. When mice were injected with collagen for 21 days when and administered flavocoxid intraperitoneally for 7 days beginning on day 21 after collagen injections, there was a significant reduction of serum PGE_2 and LTB_4 levels as well as systemic and synovial fluid levels of TNFα, high mobility group box (HMGB)-1 and IL-6, while increasing IL-10 in the joint space [106]. Flavocoxid also prevented histological bone erosion in the CIA model decreasing the receptor activator of nuclear factor-κB ligand (RANKL) and increasing osteoprotegerin (OPG), reducing NFκB expression and preventing the loss of the inhibitor of NFκB, IκBα in the joint.

These results suggest that flavocoxid is able to modulate the inflammatory aspects of joint disease in preclinical models. Though preclinical, animal studies can provide directional guidance for development of a product for OA and other disease states, human clinical trials are necessary to determine the therapeutic safety and efficacy.

Flavocoxid Clinical Safety and Efficacy:

A single-dose pharmacokinetic study of 500 mg was given to 10 healthy individuals to determine uptake and excretion of the baicalin component of flavocoxid [107]. Although considerable individual variation was noted in the data, the average AUC was 7,007 µg/ml/hour, Cmax was 0.93 µg/ml, Tmax was 5.8 hours, and the T ½ was approximately 11-12 hours for baicalin. Food intake reduced baicalin absorption by about 10%. The half-life for catechin has been reported to range from 2 to 3 hours [108] and plasma levels are observed to peak ~3 h after administration in

humans, with levels still detectable 120 h later, probably due to reabsorption and enterohepatic circulation [109].

The mechanism of flavocoxid modulation of *peroxidase* activity of the COX enzymes would suggest that this agent would not interfere with platelet function. When healthy, human volunteers (n=10) took flavocoxid (500 mg BID) for 14 days, there was no significant change in either AA-induced or spontaneous platelet aggregation or bleed times compared to baseline [66]. Serum concentrations of TxA_2 were unaffected correlating with the platelet function study. These results suggest that flavocoxid does not change TxA_2-induced platelet aggregation although it inhibits COX-1 *peroxidase* activity *in vitro*. Finally, flavocoxid did not inhibit or potentiate the anti-coagulant effect of warfarin, as measured by INR, in previously warfarinized OA patients at either 250 or 500 mg BID after 14 days of dosing (n=59) [66]. In the same manuscript, a preclinical model of aspirin-induced bleeding showed that flavocoxid neither augmented or inhibited bleed times in combination with aspirin or alone. Additional safety and efficacy studies in OA subjects have also recently been performed.

In an 8-week randomized, placebo-controlled human clinical trial (n=80), flavocoxid administered to healthy volunteers at 250 mg QD demonstrated an equivalent, mild adverse event (AE) profile to placebo, with no changes in serology or blood chemistry in healthy individuals [102]. Morgan et al [110] enrolled subjects (n=59) with moderate to severe OA [Kellgren-Lawrence (K-L) 2-3 scores] and "below average" to "moderately above average cardiovascular risk" subjects as judged by Framingham Criteria to examine the effects of 250 mg BID of flavocoxid in a randomized, placebo-controlled single-site study for 12 weeks. After 12 weeks, there were no changes in blood chemistries, but flavocoxid showed a significantly reduced number of upper respiratory AEs (p<0.0003) compared to placebo suggesting and anti-infective and anti-leukotriene effect. Both baicalin and catechin have anti-viral and antibacterial activities against a variety of pathogens and are used for this purpose as approved drugs in Asia [111-115]. The significant reduction in upper respiratory AEs may also suggest a beneficial role for 5-LOX inhibition in modulating vasoconstrictive LTs in the upper airways. There were also no differing anxiolytic effects in the two groups in the study. After showing an acceptable safety profile in both animals and humans at lower doses, flavocoxid was then tested for safety and efficacy at 500 mg BID.

Levy et al [116] showed that when flavocoxid was administered at 500 mg BID versus 500 mg BID naproxen during a 4-week, double-blind prospective short-term acute therapy study (n=103) in moderate to severe OA subjects (K-L 2-3 scores), it showed equivalent efficacy to naproxen as measured by the short Western Ontario and McMaster Osteoarthritis Index (WOMAC) as well as by patient and physician visual analogue scale (VAS) scores (Table 2) [From 116]. Adverse events were mild and roughly equivalent in both arms of this short-term efficacy study. The trial was not powered sufficiently to judge safety. Therefore, a longer and larger trial was performed in patients with OA of the knee using validated measures to assess long-term safety and efficacy.

This study was a randomized, multi-center, double-blind study in which 220 subjects who had been on previous NSAID therapy using a flare design after washout were administered either flavocoxid (500 mg BID) or naproxen (500 mg BID) to measure long-term safety and efficacy over a 12-week period [117]. Outcome measures included WOMAC, timed walk, investigator

and subject measures for disease activity, global response to therapy assessed by investigator and subject and subject VASs for discomfort, overall disease activity and index joint tenderness and mobility. More than 90% of the subjects in both groups noted significant reduction in the signs and symptoms of knee OA with no statistically significant differences in efficacy between the flavocoxid and naproxen groups when the entire intent to treat population was analyzed for any parameter (Table 3). Trends toward significance were noted in 6 out of 7 efficacy measures for flavocoxid over naproxen between 6 and 12 weeks, with subject global response to therapy (SGRT) being the only exception.

Table 2. Improvement in Western Ontario and McMaster University Index (WOMAC) and visual analogue scale (VAS)

	Flavocoxid 500 mg BID (n = 52)		Naproxen 500 mg BID (n = 51)		Within-group	Between-group
	Patients with improvement	Improvement	Patients with improvement	Improvement		
	n (%)	Mean (±SEM)	n (%)	Mean (±SEM)	P	P
WOMAC	41 (79%)	32% (33)	45 (88%)	36% (36)	<.001	.67
PGAD	43 (83%)	31% (32)	38 (75%)	38% (35)	<.001	.34
SGAD	45 (87%)	162% (249)	45 (88%)	130% (188)	<.001	.46
SGADc	45 (87%)	28% (39)	45 (88%)	38% (39)	≤.001	.20

Fisher exact test: approximately 85% of both flavocoxid and naproxen groups showed improvement.
Western Ontario and McMaster University Osteoarthritis Index (WOMAC), Physician Global Assessment of Disease Visual Analogue Scale (PGAD), Subject Global Assessment of Disease Visual Analogue Scale (SGAD), Subject Global Assessment of Discomfort Visual Analogue Scale (SGADc) [From 116].

Trends for efficacy observed in the formal, long-term safety and efficacy trial [117] between 6 and 12 weeks initiated further investigation into the subpopulation effects of flavocoxid. When the subjects below the mean for the investigator global assessment of disease (IGAD <8) were analyzed for the WOMAC composite index and its subscales, flavocoxid showed statistically better scores at 6 and 12 weeks for the WOMAC index and the subscales of pain and physical function suggesting moderate OA subjects respond better to flavocoxid than to naproxen [118]. Further subanalysis of subjects with walking times less than 14 seconds showed that flavocoxid seemed to become more efficacious compared to naproxen with p values improving between 6 and 12 weeks. The reason for the longer onset of action and continued improvement may be due to the pharmacodynamics of flavonoid binding to and absorption into red blood cells which requires saturation before full efficacy may be attained [119].

In terms of safety, flavocoxid exhibited significantly fewer upper GI and renal (edema) AEs [117]. Over 12-weeks, there was a mean increase of 0.015 mg/dL in serum creatinine in the naproxen arm and a mean decrease of 0.018 mg/dL in the flavocoxid arm (P=0.18) suggesting a possible trend toward less renal toxicity with flavocoxid. Twenty-four subjects had elevations of hepatic transaminase enzymes greater than 1.2 the upper limit of normal at screening (13 flavocoxid; 11 naproxen). At 12 weeks, approximately half of these had normalized (7 flavocoxid; 5 naproxen). Conversely, there were 16 subjects whose transaminases were normal at screening and elevated at 12 weeks (11 flavocoxid; 5 naproxen [p=0.087]). There were 12 elevations of total bilirubin between screening and 12 weeks (5 flavocoxid; 7 naproxen [p=0.18]). There were only 2 subjects with elevated alkaline phosphatase at screening, one in each group. Almost all of the abnormalities for hepatic transaminases and bilirubin in both

groups were within 1.5-3 times upper limit of normal for the reference laboratory and none exceeded 5-times the upper limit of normal [117]. Because of the fluctuating numbers of minor transaminase abnormalities, it is unclear if these data are related to study products or due to environmental or cultural phenomena.

Table 3. Summary of efficacy parameters outcomes for flavocoxid and naproxen at 6 and 12 weeks [From 117]

	Baseline, mean (SEM)	Week 6, mean (SEM)	Week 12, mean (SEM)	*P* value (6 weeks)	*P* value (12 weeks)
WOMAC index*					
Flavocoxid	40.55 (1.35)	59.68 (1.67)	66.47 (1.80)	0.74	0.19
Naproxen	41.46 (1.17)	61.32 (1.70)	65.86 (1.86)		
WOMAC: pain*					
Flavocoxid	43.21 (1.36)	63.07 (1.80)	70.09 (1.36)	0.62	0.35
Naproxen	43.55 (1.33)	64.56 (1.78)	68.38 (1.96)		
WOMAC: stiffness*					
Flavocoxid	38.44 (1.94)	56.96 (2.10)	63.80 (2.20)	0.79	0.69
Naproxen	39.91 (1.78)	60.31 (2.09)	66.34 (2.230		
WOMAC: physical function*					
Flavocoxid	40.01 (1.40)	59.00 (1.66)	65.72 (1.80)	0.96	0.13
Naproxen	41.02 (1.19)	60.49 (1.72)	65.07 (1.84)		
SGRT†					
Flavocoxid	NA	5.94 (0.20)	6.39 (0.20)	0.06	0.43
Naproxen	NA	5.89 (0.17)	6.26 (0.16)		
IGRT‡					
Flavocoxid	NA	5.95 (0.19)	6.39 (0.20)	0.67	0.41
Naproxen	NA	5.85 (0.17)	6.31 (0.15)		
Timed walk (seconds)					
Flavocoxid	16.07 (0.56)	13.51 (0.56)	12.61 (0.59)	0.21	0.08
Naproxen	15.39 (0.46)	13.20 (0.48)	12.43 (0.47)		

*(0=worst, 100=best); †(0=no change, 10=excellent); ‡(0=none, 10=outstanding).
IGRT=Investigator Global Response to Therapy; NA=not applicable; SEM=standard error of the mean; SGRT=Subject Global Response to Therapy; WOMAC=Western Ontario and McMaster Universities Osteoarthritis Index.

Post-marketing surveillance tracked for more than 8 years of marketing Limbrel (flavocoxid) in the US and Puerto Rico has found a low incidence of reported elevated liver function tests (LFT) (40 events out of almost 325,000 patients or 0.012%) [120]. Most of these LFTs were 1-3x's the upper limit of normal, but a few notable cases were severe hepatic reactions accompanied by jaundice, some with eosinophilia suggesting an allergic basis. All abnormal LFT appeared in the first three months of flavocoxid administration and all resolved without residua after discontinuing flavocoxid. By comparison, the incidence of post-marketing reports of LFTs for NSAIDS is between 2.7% and 3% worldwide [121].

   In a recent case study from a registry of 877 individuals drug induced liver injury accumulated over a 6 year period with 3 patients in the registry had liver injury linked to

flavocoxid use with one patient possibly linked to use of the product [122]. Abnormal LFT appeared 1 to 3 months after beginning flavocoxid therapy in the individuals which were identified. After discontinuing flavocoxid, all patients fully recovered without residua unlike other anti-inflammatory agents which have been shown to cause liver failure, in some cases requiring liver transplantation [123, 124]. As shown above in a head-to-head clinical trial, flavocoxid had the same incidence of LFT as naproxen. All LFTs appear to be idiosyncratic with no known causality. Therefore, it is recommended that liver function be tested two months after initiating therapy on flavocoxid and then thereafter as per usual practice of the physician.

Hypersensitivity pneumonitis is the only other serious AE that has been reported in post-marketing surveillance for flavocoxid. To date, there have been 12 confirmed and 5 unconfirmed cases of this event in post-marketing surveillance [120]. These have occurred in patients with no prior history of allergic or pulmonary disease or other predictive factors and appear to be entirely idiosyncratic. Youssef and Tomic [125] noted a single case of **"bibasilar infiltrates and CT chest confirmed bilateral lower lobe consolidation"** in a 64-year old female patient. The pneumonitis resolved with oxygen administration, corticosteroids and discontinuation of flavocoxid without residua. These serious AEs have been reported to the US Food and Drug Administration MedWatch [126].

It is always helpful to assess safety and efficacy in a real world clinical setting. A post-marketing, phase IV-like study of flavocoxid at 500 mg BID (n=1067) conducted in 41 rheumatology practices across the U.S. over a 2-month period was performed in moderate to severe OA patients (K-L 2-3 scores) [127]. There was significant improvement in 66% of patients using physician administered VAS pain scales. The strongest efficacy was noted in those patients with moderate to severe OA (Figure 6A), in those patients who were non-responders to previous NSAIDs for OA (Figure 6B) and in males.

Out of 1067 individuals initially enrolled in the study, 1005 persons completed all scheduled visits. Six dropped out because of an AE [bladder burning (1), sour stomach (1), increased pain (1), severe diarrhea (1), and unspecified (2)] while 56 individuals were lost to follow-up. Drop-out rates were much lower (0.6%) compared to a similar post-marketing studies of diclofenac (18%) and celecoxib (13% total withdrawals with 4% withdrawing due to GI AEs) [128, 129]. A low incidence of AEs (~10%) and good overall tolerability of patients to flavocoxid was noted in this tightly monitored study. The use of flavocoxid also improved upper GI tolerability in almost 50% of previous NSAID-intolerant users and reduced therapy interruption in 90% of participants with a history of NSAID-induced, GI-related therapy interruptions. Importantly, the use of proton pump inhibitors (PPIs) or histamine-2 receptor blockers (H2s) decreased or ceased by over 30% in patients who previously required them in order to tolerate their prior NSAID therapy.

The clinical trials and phase-IV study cited above, in addition to national rheumatologist opinion, and literature comparisons were utilized to perform a pharmacoeconomic analysis. This analysis demonstrated that the cost of care, using a "one year window of treatment" decision model to estimate the total costs associated with use of flavocoxid or naproxen for patients with mild to moderate OA over age 65, that Limbrel (flavocoxid) was lower in the flavocoxid treated patients in the first year of therapy [130]. Thus, even though generic NSAIDs may have a cheaper initial cost, when the cost of gastroprotective medications and side effect treatment,

including hospitalizations for upper GI events are taken into account, flavocoxid is the safer and less expensive option.

A

B
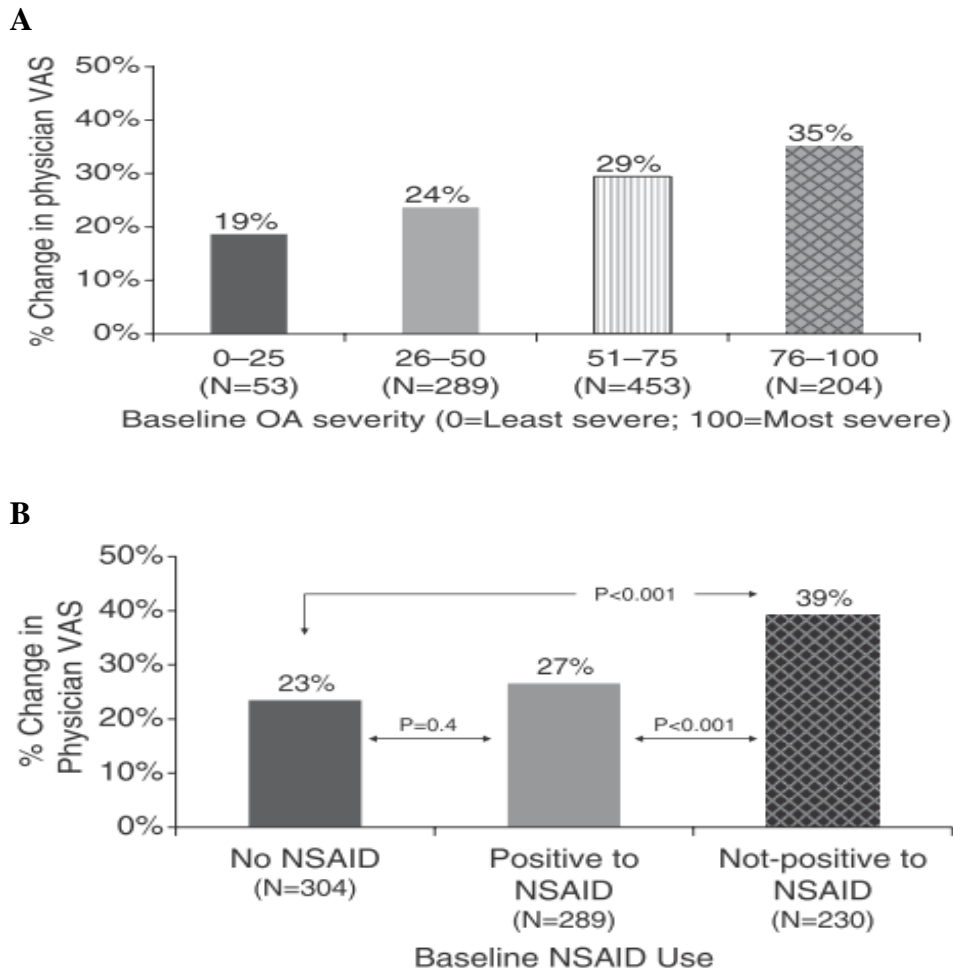
Figure 6. Percent improvement in Physician Global Assessment of Disease (PGAD) visual analog scales (VAS)-stratified by baseline OA severity in patients administered 500 mg BID Limbrel® for 8 weeks. All between-group differences and each group's improvement over baseline was significant, p<0.001 (A). Percent improvement in PGAD VAS-based on baseline NSAID use and response to this prior use in patients administered 500 mg BID Limbrel® for 8 weeks. All groups also reported a significant improvement over baseline, p<0.001 (B) [From 127].

Finally, a recent case study report suggested how Limbrel can be used in patients with drug failures and toxicities while on traditional NSAIDs and in those with cardiovascular comorbidity associated with the utilization of selective COX-2 inhibitors [131]. A 66-year old female with congestive heart failure on an angiotensin-converting enzyme (ACE) inhibitor, a beta blocker, and thiazide diuretic also having type 2 diabetes mellitus treated with metformin and sulfonylurea and mild renal insufficiency and who had a GI ulceration due to naproxen as well as mild hypertension was administered 500 mg BID of flavocoxid for three months. She noted a decrease in dyspepsia and her blood pressure dropped from 142/92 to 134/84. Her OA

symptoms and edema improved over the three months of therapy suggesting the utility of flavocoxid in this fragile patient type.

Other Nutritional Molecules/Formulations:

Nutritional intervention in the form of medical foods, functional foods and dietary supplements is more commonplace due to patient perception and reported side effects of current NSAIDs. A number of nutritional molecules have been tested in clinical trials for OA. Pycnogenol® is a mixture of procyanidins, flavonoids and organic acids extracted from French maritime pine bark [132]. Curcumin has been complexed with soy derived phosphatidylcholine (Meriva®) to create a more bioavailable form of the molecule [133]. Another bioavailable form of curcumin, BCM-95® CG (Biocurcumax™), contains curcumoids as well as tumerone oil co-purified from *Curcuma longa* [134]. Three different forms of boswellic acid exist from *Boswellia serrata*, BosPure® a 10% or more purity 3-O-acetyl-keto-11-beta-boswellic acid (AKBA) and very low β-boswellic acid (<5%) extract, 5-Loxin®, a 30% AKBA containing extract [135] and Aflapin® a 30% AKBA containing extract with *B. serrata* non-volatile oils [144]. Sterol-rich fractions of avocado soybean unsaponifiables (ASU) have been isolated as early has the 1970's for use in clinical medicine [136]. A comparison of different nutritional molecules used in safety and efficacy OA trials are listed in Table 4.

Table 4. Clinical studies of nutritional molecules in osteoarthritis

Reference	Type of Study	Intervention	Participants	Follow-up and Assessment(s)	Major Safety and Efficacy Outcome(s)
Flavocoxid (Limbrel®)					
102	RCT, double-blind, placebo controlled safety	250 mg QD of Limbrel vs placebo	80 healthy subjects	8 week study, assessments at baseline and 8 weeks	Mild AE profile similar to placebo, No changes in serology or blood chemistry in either group
110	RCT, double-blind, placebo-controlled safety	250 mg BID Limbrel® vs placebo BID	59 subjects, moderate to severe OA (K-L=2-3), below average to moderately above average CV risk	12 week study, assessments at baseline, 2, 4, 8, and 12 weeks	No differences in blood chemistries, Significantly reduced upper respiratory AEs for flavocoxid vs placebo (p<0.0003), No differences in depression or anxiety scores
116	RCT, double-blind, direct comparator to naproxen for efficacy	500 mg BID Limbrel® vs 500 mg BID naproxen	103 subjects, moderate to severe OA (K-L=2-3)	4 week study, assessments at baseline and 4 weeks	Equivalent AEs, Equivalent short WOMAC, subject/physician VAS scores
117, 118	RCT, double-blind, direct comparator to naproxen for safety and efficacy	500 mg BID Limbrel® vs 500 mg BID naproxen	220 subjects, moderate to severe OA (K-L=2-3)	12 week study, assessments at baseline, 6 and 12 weeks	Statistically better upper GI (dyspepsia) and renal (edema) AE profile for flavocoxid vs naproxen, Statistically more flatulence for flavocoxid vs naproxen, Equivalent WOMAC composite and subscale scores as well as various subject/physician VAS scores and

					timed walk in the entire intent to treat population, Statistically better WOMAC composite, pain and physical function subscales as well as various subject/physician VAS scores and timed walk of flavocoxid vs naproxen in moderate OA subjects
127	Open-Label, Phase IV Post-marketing, safety with physician judged efficacy	500 mg BID Limbrel®	1067 patients with moderate to severe OA (K-L=2-3)	8 week study, assessments at baseline and 8 weeks	~10% AE rate, 31% reduction in gastroprotective use, 48% greater GI tolerance in patients previously with GI problems with NSAIDs, Statistically better pain response rates in patients who had no previously responded to NSAID therapy and in patients with the highest baseline VAS scores
131	Open-Label, Case Study, safety with physician and patient judged efficacy	500 mg BID Limbrel®	1 patient with severe OA, congestive heart failure, mild hypertension, type 2 diabetes mellitus, mild renal insufficiency, and a previous GI ulcers on NSAIDs	3 month follow-up by physician	The patient showed a drop in blood pressure toward normal, improved edema and returned to her exercise routine. There were no other complaints while on Limbrel.
Pycnogenol®					
137	RCT, double-blind, placebo-controlled safety and efficacy	150 mg QD Pycnogenol	100 subjects with mild to moderate OA (K-L=1-2)	3 month study, assessments at clinic at baseline, 4, 8 and 12 weeks of therapy with a 4 week post-intervention assessment, weekly VAS pain scale and biweekly WOMAC composite index and subscales by subjects	Statistically significant improvement for WOMAC composite index and its subscales by 8 weeks for Pycnogenol® vs placebo, No statistical difference in VAS scores or AEs, Trend for decreased rescue analgesic use in the Pycnogenol® vs placebo group
138	RCT, double-blind, placebo-controlled safety and efficacy	100 mg QD	145 subjects with mild to moderate OA (K-L=1-2)	3 month study, assessments at baseline and 3 months	Statistically significant improvement for WOMAC composite index for Pycnogenol® vs placebo, Statistical improvement in edema for for Pycnogenol® vs placebo, Statistical reduction in

					rescue analgesic use in the for Pycnogenol® vs placebo group
Curcumin (Meriva®)					
139	Open-label, placebo comparison recruited from registry of patients with vascular disease for efficacy	1 g QD Meriva® (200 mg curcumin)	50 patients with mild to moderate OA (K-L=1-2)	3 month study, assessments at baseline, 2 and 3 months	Statistically significant improvement of WOMAC composite index for Meriva® plus standard of care vs standard of care alone at 3 months, Significantly better physical performance in walking on treadmill at 2 and 3 months for Meriva® plus standard of care vs standard of care alone, Significant reduction in CRP in Meriva® plus standard of care vs standard of care alone at 2 months, Decreased cost of treatment (rescue analgesics, treatment, hospitalization) in Meriva® plus standard of care vs standard of care alone, No difference between Meriva® plus standard of care vs standard of care alone in social or emotional function scores
140	Open-label, placebo comparison recruited from registry of patients with vascular disease for efficacy	500 mg BID Meriva® (200 mg curcumin)	100 patients with mild to moderate OA (K-L=1-2)	8 month study, assessments at baseline and 8 months	Statistically significant improvement for WOMAC composite index and subscales of pain, stiffness and physical function for Meriva® plus standard of care vs standard of care alone, Significant improvement in social and emotional function for Meriva® plus standard of care vs standard of care alone, Significantly better physical performance in walking on treadmill for Meriva® plus standard of care vs standard of care alone, Significantly improved levels of the markers sCD40L, IL-1b, IL-6, sVCAM-1, and ESR for Meriva® plus standard of care vs standard of care
BCM-95®					
141	RCT, double-blind, direct comparator to celecoxib for safety and efficacy	500 mg BID BCM-95® with BosPure® (Rhulief™) vs 100 mg BID celecoxib	30 subjects diagnosed with OA of the knee, unknown severity	12 week study, assessments at baseline and 12 weeks	Equivalent pain scores, walking distance and range of motion between groups, Both groups showed statistical improvement over baseline, Equivalent AEs
5-Loxin®					
142	RCT, double-blind, placebo-controlled safety and efficacy	100 mg QD and 250 mg QD 5-Loxin® vs placebo	75 subjects with mild to moderate OA (According to baseline VAS scoring)	90 day study, assessments at baseline, 7, 30, 60 and 90 days	Both doses of 5-Loxin® showed statistically significant improvements in VAS, WOMAC composite index and subscale scores for pain, stiffness and physical function as well as scores for the

					Lequesne's Functional Index [143] vs placebo starting at 7 days for the high dose, the 5-Loxin® also showed statistically decreased levels of metalloproteinase-3 (MMP-3) from synovial fluid compared to placebo, AEs were comparable in all groups
144	RCT, double-blind, comparator, placebo-controlled safety and efficacy	100 mg QD 5-Loxin® vs 100 mg QD Aflapin® vs placebo	60 subjects with mild to moderate OA (According to baseline VAS scoring)	90 day study, assessments at baseline, 7, 30, 60 and 90 days	Both 5-Loxin® and Aflapin® showed statistically significant improvements in VAS, WOMAC composite index and subscale scores for pain, stiffness and physical function as well as scores for the Lequesne's Functional Index [143] vs placebo, Onset of action was quicker for Aflapin, AEs were comparable in all groups
Avocado/Soy bean Unsaponifi-ables (ASU)					
145	RCT, double-blind, placebo-controlled safety and efficacy, added on to NSAID therapy	300 mg QD ASU vs placebo	164 subjects with mild to moderate OA (K-L=1-3), using NSAIDs	3 month study, assessments at baseline, 45 and 90 days, NSAID co-administered for first 45 days, reduction in NSAID use measured thereafter	Statistical reduction in NSAID use in ASU group vs placebo, Global quality of life scores statistically better in ASU vs placebo, No difference in pain scores, Comparable AEs in both groups
146	RCT, double-blind, placebo-controlled safety and efficacy, added on to NSAID therapy	300 mg and 600 mg QD ASU vs placebo	260 subjects with mild to moderate OA (K-L=1-3), using NSAIDs	3 month study, assessments at baseline, 30, 60 and 90 days, NSAID co-administered for first 30 days, reduction in NSAID use measured thereafter	Statistical reduction in NSAID in ASU group vs placebo, Significantly better Lequesne's Functional Index [143] and pain scores in ASU groups vs placebo, Global quality of life scores statistically better in ASU groups vs placebo, Comparable AEs in all groups
147	RCT, double-blind, placebo-controlled safety and efficacy, added on to NSAID therapy	300 mg QD ASU vs placebo	164 subjects with mild to moderate OA (K-L=1-3), using NSAIDs	8 month study with a 2 month follow up, NSAIDs withdrawn 15 days prior to start of trial and then allowed as rescue medication	Statistical improvement Lequesne's Functional Index [143] and pain scores in ASU group vs placebo, Significantly better functional ability and global quality of life scores in ASU vs placebo, Non-statistical reduction in NSAID use in ASU group vs placebo, Improvements in functional and quality of life scores continued 2 months after discontinuation vs placebo, Comparable AEs in both groups

Randomized Controlled Trial (RCT), Western Ontario and McMaster University Osteoarthritis Index (WOMAC), Twice daily (BID), Osteoarthritis (OA), Adverse Events (AEs), Gastrointestinal (GI), Kellgren-Lawrence (K-L), Nonsteroidal Anti-inflammatory Drugs (NSAIDs), Visual Analogue Scale (VAS), Soluble CD40 ligand (sCD40L), Interleukin 1beta (IL-1β), Interleukin 6 (IL-6), Soluble vascular cell adhesion molecule-1 (VCAM-1), Erythrocyte Sedimentation Rate (ESR), Avocado Soybean Unsaponifiables (ASU)

Lastly, the intake of glucosamine and chondroitin is controversial and beyond the scope of this review. Some clinical studies [148-150] and meta-analyses [151] suggest effectiveness in OA while other meta-analyses contradict these data [152, 153].

CONCLUSION:

Flavocoxid is arguably one of the better studied nutritional therapies for OA with a history of use in the US since 2004. Though reasonable and promising data exist for other nutritional interventions, consistency of formulation and mixtures with other agents make their choice difficult to recommend by physicians and confusing to consumers. Since medical foods are assessed for GRAS by expert panel review of animal and human toxicology, have known and FDA-auditable quantities of specific nutrients as well as a requirement they be produced under GMPs, have clear instructions of use in the form of package inserts which must document their claims of safety and efficacy and are required to be dosed and monitored by physicians to assure their safe use, flavocoxid represents a safe and reasonable option for patients with OA who have shown toxicities to NSAIDs or have co-morbidities which require polypharmacy. Additional studies in special populations such as renal insufficient individuals and patients with a history of GI ulceration are needed to verify flavocoxid's safety profile. In addition, larger, future studies in OA of the knee, hip and hand will further expand the knowledge and use of flavocoxid in patients with chronic joint disease.

List of Abbreviations:

5-LOX-5-lipoxygenase, AA-arachidonic acid, ACE-angiotensin-converting enzyme inhibitor, AE-adverse event, ASU-avocado soybean unsaponifiables, BID twice daily, CLP-cecal ligation and puncture, COX-cyclooxygenase, CIA-collagen-induced arthritis, CRP-C-reactive protein, DMD-Duchene muscular dystrophy, DPPH-2,2-di(4-tert-octylphenyl)-1-picrylhydroxyl, ESR-erythrocyte sedimentation rate, FRAP-ferric reducing/antioxidant power, GRAS- generally recognized as safe, GI-gastrointestinal, GMPs-good manufacturing practices, H2s-histamine-2 receptor blockers, HNE- 4-hydroxynonenal, HORAC-hydroxyl radical absorbance capacity, IGRT-Investigator Global Response to Therapy, IL-1β-Interleukin-1β, IL-6-Interleukin 6, IL-10-Interleukin 10, HMGB-1- high mobility group box, iNOS-inducible nitric oxide synthase, INR-international normalized ratio, Kellgren-Lawrence-K-L, LFT-liver function tests, LT-Leukotriene, MAPKs-mitogen-activated protein kinases, MDA-malondialdehyde, MMP-3-metalloproteinase-3, MOA-mechanism of action, NFκB-nuclear factor kappa B, NO-Nitric oxide, NOAEL-no-observed adverse-effect-level, NSAIDs-nonsteroidal anti-inflammatory drugs, OA-osteoarthritis, PGAD-Physician Global Assessment of Disease Visual Analogue Scale, PGE2-prostaglandin E2, PGG2-prostaglandin G2, PGH2-prostaglandin H2, PGI2-prostacyclin, PGs-Prostaglandins, PLA2-Phospholipase A2, PPIs-proton pump inhibitors, RA-rheumatoid arthritis, RANKL-receptor activator of nuclear factor-κB ligand, SGAD-Subject

Global Assessment of Disease Visual Analogue Scale, SGADc-Subject Global Assessment of Discomfort Visual Analogue Scale, SGRT-Subject Global Response to Therapy, sVCAM-1-soluble vascular cell adhesion molecule, SORAC-superoxide radical averting capacity, TEAC-trolox equivalent antioxidant capacity, TNFα-Tumor necrosis factor-alpha, TxA$_2$-Thromboxane, VAS-visual analogue scale, WOMAC-Western Ontario and McMaster Osteoarthritis Index, RCT-randomized, placebo-controlled trial

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REFERENCES:

1. Sanchez-Borges M, Caballero-Fonseca F, Capriles-Hulett A, Gonzalez-Aveledo L: Hypersensitivity reactions to nonsteroidal anti-inflammatory drugs: an update. Pharmaceuticals 2010, 3:10-18.
2. Martel-Pelletier J, Mineau F, Fahmi H, Laufer S, Reboul P, Boileau C, Lavigne M, Pelletier JP: Regulation of the expression of 5-lipoxygenase-activating protein/5-lipoxygenase and the synthesis of leukotriene B(4) in osteoarthritic chondrocytes: role of transforming growth factor beta and eicosanoids. Arthritis and Rheumatism 2004, 50(12):3925-33.
3. Burnett BP, Levy R, Cole BJ: Metabolic mechanisms in the pathogenesis of osteoarthritis. A review. J Knee Surg 2006, 19(3):191-7.
4. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, Gabriel S, Hirsch R, Hochberg MC, Hunder GG, Jordan JM, Katz JN, Kremers HM, Wolfe F; National Arthritis Data Workgroup: Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum 2008, 58(1):26-35.
5. Andersen RE, Crespo CJ, Ling SM, Bathon JM, Bartlett SJ: Prevalence of significant knee pain among older Americans: results from the Third National Health and Nutrition Examination Survey. J Am Geriatr Soc 1999, 47(12):1435-8.
6. Dillon CF, Rasch EK, Gu Q, Hirsch R: Prevalence of knee osteoarthritis in the United States: arthritis data from the Third National Health and Nutrition Examination Survey 1991-94. J Rheumatol 2006, 3(11):2271-9.
7. Grotle M, Hagen KB, Natvig B, Dahl FA, Kvien TK: Prevalence and burden of osteoarthritis: results from a population survey in Norway. J Rheumatol 2008, 35(4):677-84.
8. Woolf AD, Pfleger B: Burden of major musculoskeletal conditions. Bull World Health Organ 2003, 81(9):646-56.
9. Leaf A, Weber PC: A new era for science in nutrition. Am J Clin Nutr 1987, 45:1048-1053.

10. Update to Orphan Drug Act of 1988, 21 USC Section 360ee(b)(3).

11. Nutritional Labeling and Education Act of 1993, 21 CFR Section 101.9(j)(8).

12. Peterson RO, Vucetich JA, Fenton G, Drummer TD, Larsen CS: Ecology of arthritis. Ecol Lett 2010, 13(9):1124-8.

13. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, Brand-Miller J: Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 2005, 81(2):341-54

14. Sprecher HW, Baykousheva SP, Luthria DL, Mohammed BS: Differences in the regulation of biosynthesis of 20- versus 22-carbon polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids 1995, 52(2-3):99-101.

15. Lippiello L: Lipid and cell metabolic changes associated with essential fatty acid enrichment of articular chondrocytes. Proc Soc Exp Biol Med 1990, 195(2):282-227.

16. Lippiello L, Walsh T, Fienhold M: The association of lipid abnormalities with tissue pathology in human osteoarthritic articular cartilage. Metabolism 1991, 40(6):571-576.

17. Plumb MS, Aspden RM: High levels of fat and omega-6 fatty acids in cancellous bone in osteoarthritis. Lipids Health Dis 2004, 3:12.

18. Wang Y, Wluka AE, Hodge AM, English DR, Giles GG, O'Sullivan R, Cicuttini FM: Effect of fatty acids on bone marrow lesions and knee cartilage in healthy, middle-aged subjects without clinical knee osteoarthritis. Osteoarthritis Cartilage 2008, 16(5):579-83.

19. Xu H, Watkins BA, Adkisson HD: Dietary lipids modify the fatty acid composition of cartilage, isolated chondrocytes and matrix vesicles. Lipids Health Dis 1994, 29:619-625.

20. Hart DJ, Doyle DV, Spector TD: Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. J Rheumatol 1995, 22(6):1118-1123.

21. Baker KR, Matthan NR, Lichtenstein AH, Niu J, Guermazi A, Roemer F, Grainger A, Nevitt MC, Clancy M, Lewis CE, Torner JC, Felson DT: Association of plasma n-6 and n-3 polyunsaturated fatty acids with synovitis in the knee: the MOST study. Osteoarthritis Cartilage 2012, 20(5):382-7.

22. Gruenwald J, Petzold E, Busch R, Petzold HP, Graubaum HJ: Effect of glucosamine sulfate with or without omega-3 fatty acids in patients with osteoarthritis. Adv Ther 2009, 26(9):858-71.

23. Jacquet A, Girodet PO, Pariente A, Forest K, Mallet L, Moore N: Phytalgic©, a food supplement, vs placebo in patients with osteoarthritis of the knee or hip: a randomized double-blind placebo-controlled clinical trial. Arthritis Res Ther 2009, 11(6): R192.

24. Middleton E Jr, Kandaswami C, Theoharides TC: The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000, 52(4):673-751.

25. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T, Aromaa A: Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 2002, 76(3):560-8.

26. Yoon JH, Baek SJ: Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei Med J 2005, 46(5):585-96.

27. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, et al: Flavonoid intake and long-term risk of

coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995, 155(4):381-6.

28. Beecher GR: Overview of Dietary Flavonoids: Nomenclature, Occurrence and Intake. J Nutr 2003, 133:3248S-3254S.

29. Sanchez-Moreno C, Cano MP, de Ancos B, Plaza L, Olmedilla B, Granado F, Martín A: Consumption of high-pressurized vegetable soup increases plasma vitamin C and decreases oxidative stress and inflammatory biomarkers in healthy humans. J Nutr 2004, 134:3021-3025.

30. Chun OK, Chung SJ, Song WO: Estimated dietary flavonoid intake and major food sources of US adults. J Nutr 2007, 137:1244-1252.

31. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventós RM, Berenguer T, Jakszyn P, Barricarte A, Ardanaz E, Amiano P, Dorronsoro M, Larrañaga N, Martínez C, Sánchez MJ, Navarro C, Chirlaque MD, Tormo MJ, Quirós JR, González CA: Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). J Am Diet Assoc 2010, 110(3):390-8.

32. Chang AS, Yee Yeong BY, Koh WP: Symposium on Plant Polyphenols: Nutrition, Health and Innovations, June 2009. Nutr Rev 2010, 68(4):246-252.

33. Pomerleau J, Lock K, McKee M, Altmann DR: The challenge of measuring global fruit and vegetable intake. J Nutr 2004, 134:1175-80.

34. Wang Y, Chung SJ, Song WO, Chun OK: Estimation of daily proanthocyanidin intake and major food sources in the U.S. diet. J Nutr 2011, 141(3):447-52.

35. Gierman LM, van der Ham F, Koudijs A, Wielinga PY, Kleemann R, Kooistra T, Stoop R, Kloppenburg M, van Osch GJ, Stojanovic-Susulic V, Huizinga TW, Zuurmond AM: Metabolic stress-induced inflammation plays a major role in the development of osteoarthritis in mice. Arthritis Rheum 2012, 64(4):1172-81.

36. Chun OK, Chung SJ, Claycombe KJ, Song WO: Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. J Nutr 2008, 138(4):753-60.

37. di Giuseppe R, Di Castelnuovo A, Centritto F, Zito F, De Curtis A, Costanzo S, Vohnout B, Sieri S, Krogh V, Donati MB, de Gaetano G, Iacoviello L: Regular consumption of dark chocolate is associated with low serum concentrations of C-reactive protein in a healthy Italian population. J Nutr 2008, 138(10):1939-45.

38. Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, Hong CP, Sinaiko AR: Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. J Am Diet Assoc 2009, 109(3):414-21.

39. Landberg R, Sun Q, Rimm EB, Cassidy A, Scalbert A, Mantzoros CS, Hu FB, van Dam RM: Selected dietary flavonoids are associated with markers of inflammation and endothelial dysfunction in U.S. women. J Nutr 2011, 141(4):618-25.

40. Ahmed S: Green tea polyphenol epigallocatechin 3-gallate in arthritis: progress and promise. Res Ther 2010, 12(2):208.

41. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadev VR, Park B, Kim JH, Gupta SC, Phromnoi K, Sundaram C, Prasad S, Chaturvedi MM, Sung B: Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases:

"reverse pharmacology" and "bedside to bench" approach. Curr Drug Targets 2011, 12(11):1595-653.

42. González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martínez-Augustin O, Sánchez de Medina F: Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci Nutr 2011, 51(4):331-62.

43. Imada K, Lin N, Liu C, Lu A, Chen W, Yano M, Sato T, Ito A: Nobiletin, a citrus polymethoxy flavonoid, suppresses gene expression and production of aggrecanases-1 and -2 in collagen-induced arthritic mice. Biochem Biophys Res Commun 2008, 373(2):181-5.

44. Martin JA, Brown T, Heiner A, Buckwalter JA: Post-traumatic osteoarthritis: the role of accelerated chondrocyte senescence. Biorheology 2004, 41(3-4):479-91.

45. Kurz B, Lemke A, Kehn M, Domm C, Patwari P, Frank EH, Grodzinsky AJ, Schünke M: Influence of tissue maturation and antioxidants on the apoptotic response of articular cartilage after injurious compression. Arthritis Rheum 2004, 50(1):123-30.

46. Beecher BR, Martin JA, Pedersen DR, Heiner AD, Buckwalter JA: Antioxidants block cyclic loading induced chondrocyte death. Iowa Orthop J 2007, 27:1-8.

47. Martin JA, McCabe D, Walter M, Buckwalter JA, McKinley TO: N-acetylcysteine inhibits post-impact chondrocyte death in osteochondral explants. J Bone Joint Surg Am 2009, 91(8):1890-7.

48. McAlindon TE, Jacques P, Zhang Y, Hannan MT, Aliabadi P, Weissman B, Rush D, Levy D, Felson D: Do antioxidant micronutrients protect against the development and progression of knee osteoarthritis? Arthritis Rheum 1996, 39(4):648-56.

49. Baker K, Niu J, Goggins J: The effects of vitamin C intake on pain in knee osteoarthritis (OA). Arthritis Rheum 2003, 48:S422.

50. Wang Y, Hodge AM, Wluka AE, English DR, Giles GG, O'Sullivan R, A. Forbes A, Cicuttini FM: Effect of antioxidants on knee cartilage and bone in healthy, middle-aged subjects: a cross-sectional study. Arthritis Res Ther 2007, 9(4):R66.

51. Firuzi O, Miri R, Tavakkoli M, Saso L: Antiioxidant therapy: current status and future prospects. Curr Med Chem 2011, 18(25):3871-88.

52. Lopez HL: Nutritional interventions to prevent and treat osteoarthritis. Part I: focus on fatty acids and macronutrients. PMR 2012, 4(5 Suppl):S145-54.

53. Lopez HL: Nutritional interventions to prevent and treat osteoarthritis. Part II: focus on micronutrients and supportive nutraceuticals. PMR 2012, 4(5 Suppl):S155-68.

54. Mobasheri A, Henrotin Y, Biesalski HK, Shakibaei M: Scientific evidence and rationale for the development of curcumin and resveratrol as nutraceutricals for joint health. Int J Mol Sci 2012, 13(4):4202-32.

55. Jia Q: Generating and screening a natural product library for cyclooxygenase & lipoxygenase dual inhibitors. Studies Nat Prod Chem 2003, 29:643-718.

56. Jeong ML, Zahn M, Trinh T, Brooke FA, Ma W: Pesticide residue analysis of dietary ingredient by gas chromatography/selected-ion monitoring mass spectrometry using neutral alumina solid-phase extraction cleanup. J AOAC Internatl 2008, 90(3):630-636.

57. Zahn M, Jeong ML, Wang D, Trinh T, Brooke F, Ma W: Product-specific sample clean-up and HPLC analysis of aflatoxins for dietary product. Phytochemical Anal 2009, 20(4):335-337.

58. Brash AR: Arachidonic acid as a bioactive molecule. J Clin Invest 2001, 107:1339-1345.

59. Smith WL, DeWitt DL, Garavito RM: Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000, 69:145-182.

60. Spencer AG, Woods JW, Arakawa T, Singer II, Smith WL: Subcellular localization of prostaglandin endoperoxide H synthases-1 and -2 by immunoelectron microscopy. J Biol Chem 1998, 273:9886-9893.

61. Smith WL, DeWitt DL, Garavito RM: Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000, 69:145-182.

62. Bolego C, Buccellati C, Prada A, Gaion RM, Folco G, Sala A: Critical role of COX-1 in prostacyclin production by human endothelial cells under modification of hydroperoxide tone. FASEB J 2009, 23:605-612.

63. Ruan CH, So SP, Ruan KH: Inducible COX-2 dominates over COX-1 in prostacyclin biosynthesis: mechanisms of COX-2 inhibitor risk to heart disease. Life Sci 2011, 88:24-30.

64. Burnett BP, Jia Q, Zhao Y, and Levy RM: A medicinal extract of Scutellaria baicalensis and Acacia catechu acts as a dual inhibitor of cyclooxygenase and 5-lipoxygenase to reduce inflammation. J Medicinal Foods 2007, 10:442-451.

65. Burnett BP, Bitto A, Sqadrito F, Levy RM, Pillai L: Flavocoxid Inhibits Phospholipase A2, Peroxidase Moieties of the Cyclooxygenases (COX), 5-Lipoxygenase, Modifies COX-2 Gene Expression and Acts as an Antioxidant. Mediators Inflamm 2011, 2011:385780, published online June 22, doi: 10.1155/2011/385780.

66. Pillai L, Levy RM, Yiman M, Zhao Y, Jia Q, Burnett BP: Flavocoxid, an anti-inflammatory agent of botanical origin, does not affect coagulation or interact with anticoagulation therapies. Adv Ther 2010a, 27(6):400-11.

67. FitzGerald GA: Mechanisms of platelet activation: thromboxane A2 as an amplifying signal for other agonists. Am J Cardiol 1991, 68:11B-5B.

68. Burnett BP, Levy RM: 5-Lipoxygenase Metabolic Contributions to NSAID-Induced Organ Toxicity. Adv Ther 2012, 29(2):79-98.

69. Suzuki YJ, Forman HJ, Sevanian A: Oxidants as stimulators of signal transduction. Free Radic Biol Med 1997, 22:269-285.

70. Abramson SB: Inflammation in osteoarthritis. J Rheumatol Suppl 2004, 31:70-76.

71. Roberts L, Morrow J: The generation and actions of isoprostanes. Biochim Biophys Acta 1997, 1345:121-135.

72. Henrotin Y, Bruckner P, Pujol J: The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilag. 2003, 11:747-755.

73. Krasnokutsky S, Attur M, Palmer G, Samuels J, Abramson SB: Current concepts in the pathogenesis of osteoarthritis. Osteoarthritis Cartilage 2008, 16(Suppl 3):S1-3.

74. Hadjigogos K: The role of free radicals in the pathogenesis of rheumatoid arthritis. Panminerva Med 2003, 45(1):7-13.

75. Marcu KB, Otero M, Olivotto E, Borzi RM, Goldring MB: NF-κB Signaling: Multiple angles to target OA. Curr Drug Targets 2010, 11(5):599-613.

76. Marok R, Winyard PG, Coumbe A, Kus ML, Gaffney K, Blades S, Mapp PI, Morris CJ, Blake DR, Kaltschmidt C, Baeuerle PA: Activation of the transcription factor nuclear factor-kappa B in human inflamed synovial tissue. Arthritis Rheum 1996, 39:583-91.

77. Henrotin YE, Bruckner P, Pujol JP: The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilage 2003, 11(10):747-55.

78. Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B: Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis 2005, 64:1263-7.

79. Chandel NS, Trzyna WC, McClintock DS, Schumacker PT: Role of oxidants in NF-kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin. J Immunol 2000, 165(2):1013-1021.

80. Fernandes JC, Martel-Pelletier J, Pelletier JP: The role of cytokines in osteoarthritis pathophysiology. Biorheology 2002, 39:237-46.

81. Jacobs MD, Harrison SC: Structure of an IκBalpha/NF-κB complex. Cell 1998, 95(6):749-58.

82. Brasier AR: The NF-κB regulatory network. Cardiovasc Toxicol 2006 6(2):111-30.

83. Perkins ND. Integrating cell-signaling pathways with NF-κB and IKK function. Nat Rev Mol Cell Biol 2007, 8(1):49-62.

84. Cao G, Alessio H, Cutler R: Oxygen-radical absorbance capacity assay for antioxidants. Free Radic Biol Med 1993, 14(3):303-11.

85. Benzie IFF, Strain JJ: The ferric reducing ability of plasma as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996, 239:70-76.

86. Ou B, Hampsch-Woodill M, Flanagan J, Deemer EK, Prior RL, Huang D: Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. J Agric Food Chem 2002, 50:2772-2777.

87. Dubost NJ, Ou B, Beelman RB: Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. Food Chem 2007, 105:727-735.

88. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 1999, 26:1231-1237.

89. Sa´nchez-Moreno C: Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Technol Int 2002, 8:121-137.

90. Desai JC, Goo T, Fukata M, Sanyal S, Dikman A, Miller K, Cohen L, Brooks A, Wang Q, Abreu MT, Aisenberg J: NSAID-induced Antral Ulcers are Associated with Distinct Changes in Mucosal Gene Expression. Aliment Pharmacol Ther 2009, 30(1):71-81.

91. Tseng-Crank J, Sookyung Sung S, Jia Q, Zhao Y, Burnett BP, Park DR, Woo SS: A medicinal plant extract of *Scutellaria baicalensis* and *Acacia catechu* reduced lps stimulated gene expression in immune cells: a comprehensive genomic study using qPCR, ELISA, and microarray. J Applied Nutr 2008, 56(4):71-85.

92. Tseng-Crank J, Sookyung Sung S, Jia Q, Zhao Y, Burnett BP, Park DR, Woo SS: A medicinal plant extract of *Scutellaria baicalensis* and *Acacia catechu* reduced LPS-

stimulated gene expression in immune cells: A comprehensive genomic study using QPCR, ELISA, and microarray. J Diet Suppl 2010, 7(3):253-272.

93. Altavilla D, Bitto A, Polito F, Burnett BP, Di Stefano V, Minutoli L, Squadrito F: Flavocoxid, a dual inhibitor of cyclooxygenase and 5-lipoxygenase, blunts pro-inflammatory phenotype activation in endotoxin stimulated macrophages. Brit J Pharmacol 2009, 157:1410-8.

94. Messina S, Bitto A, Aguennouz M, Mazzeo A, Migliorat A, Polito F, Irrera N, Altavilla D, Minutoli L, Squadrito F, Vita G: Flavocoxid inhibits NF-kappaB, MAPKs and COX/5-LOX pathways and improves muscle function and morphology in *mdx* mice: a comparison study with methylprednisolone. Exp Neurol 2009, 220:349-358.

95. Safety Study of Flavocoxid in Duchene Muscular Dystrophy, ClinicalTrials.gov identifier: NCT01335295.

96. Bhatia M, Wong FL, Cao Y, Lau HY, Huang J, Puneet P, Chevali L: Pathophysiology of acute pancreatitis. Pancreatology 2005, 5:132-144.

97. Polito F, Bitto A, Irrera N, Letteria M, Fazzari C, Squadrito F, Altavilla D: Flavocoxid, a dual inhibitor of COX-2 and 5-LOX, reduces pancreatic damage in an experimental model of acute pancreatitis. Brit J Pharmacol 2010, 161:1002-1011.

98. Kirschenbaum A, Klausner AP, Lee R, Unger P, Yao S, Liu XH, Levine AC: Expression of cyclooxygenase-1 and cyclooxygenase-2 in the human prostate. Urology 2000, 56:671-676.

99. Larré S, Camparo P, Comperat E, Boulbés D, Haddoum M, Baulande S, Soularue P, Costa P, Cussenot O: Biological effect of human serum collected before and after oral intake of *Pygeum africanum* on various benign prostate cell cultures. Asian J Androl. 2011, doi:10.1038/aja.2011.132.

100. Altavilla D, Minutoli L, Polito F, Irrera N, Arena S, Magno C, Rinaldi M, Burnett B, Squadrito F, Bitto A: Effects of flavocoxid, a dual inhibitor of COX and 5-lipoxygenase enzymes, on benign prostatic hyperplasia. Br J Pharmacol 2012, 167(1):95-108.

101. Bitto A, Minutoli L, David A, Irrera N, Rinaldi M, Venuti FS, Squadrito F, Altavilla D. Flavocoxid, a dual inhibitor of COX-2 and 5-LOX of natural origin, attenuates the inflammatory response and protects mice from sepsis. Crit Care 2012, 16(1):R32.

102. Burnett BP, Silva S, Mesches MH, Wilson S, and Jia Q: Safety evaluation of a combination, defined extract of *Scutellaria baicalensis* and *Acacia catechu*. J Food Biochem 2007, 31:797-825.

103. Yimam M, Zhao Y, Maa W, Jia Q, Do SG, Shin JH: 90-Day oral toxicity study of UP446, a combination of defined extracts of *Scutellaria baicalensis* and *Acacia catechu*, in rats. Food Chem Toxicol 2010, 48:1202-9.

104. Young JM, Spires DA, Bedord CJ, Wagner B, Ballaron SJ, De Young LM: The mouse ear inflammatory response to topical arachidonic acid. J Invest Dermatol 1984, 82:367-371.

105. Burnett BP, Stenstrom KK, Baarsch MJ, Swafford WS, Ehrenzweig J, Levy RM: A flavonoid mixture, dual inhibitor of cyclooxygenase and 5-lipoxygenase enzymes, shows superiority to glucosamine/chondroitin for pain management in moderate osteoarthritic dogs. Intern J Appl Res Vet Med 2009, 7(1):1-12.

106. Polito F, Irrera N, Bitto A, Burnett BP, Marini H, Altavilla D, Squadrito F: Flavocoxid, a dual inhibitor of cox and 5-lox, attenuates inflammation and bone resorption in a mouse model of collagen induced arthritis. Submitted.

107. Levy RM, Pillai L, Burnett BP: Nutritional benefit of flavocoxid in patients with osteoarthritis: Efficacy and safety. Nutr Diet Suppl 2010c, 2:1-12.

108. Bell JR, Donovan JL, Wong R, Waterhouse AL, German JB, Walzem RL, Kasim-Karakas SE: (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. Am J Clin Nutr 2000, 71(1):103-8.

109. Hackett AM, Griffiths LA, Broillet A, Wermeille M: The metabolism and excretion of (+)-[14^C]cyanidanol-3 in man following oral administration. Xenobiotica 1983, 13(5):279-86.

110. Morgan SL, Baggott JE, Moreland L, Desmond R, Kendrach AC: The safety of flavocoxid, a medical food, in the dietary management of knee osteoarthritis. J Medicinal Foods 2009, 12(5):1-6.

111. Toda M, Okubo S, Hara Y, Shimamura T: Antibacterial and bactericidal activities of tea extracts and catechins against methicillin resistant Staphylococcus aureus. Nippon Saikingaku Zasshi 1991, 46:839-845.

112. Mabe K, Yamada M, Oguni I, Takahashi T: *In vitro* and *in vivo* activities of tea catechins against Helicobacter pylori. Antimicrob Agents Chemother 1999, 43:1788-1791.

113. Li BQ, Fu T, Dongyan Y, Mikovits JA, Ruscetti FW, Wang JM: Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. Biochem Biophys Res Commun 2000, 276:534-538.

114. Yang D, Hu H, Huang S, Chaumont JP, Millet J: Study on the inhibitory activity, in vitro, of baicalein and baicalin against skin fungi and bacteria. Zhong Yao Cai 2000, 23:272-274.

115. Yamada H, Takuma N, Daimon T, Hara Y: Gargling with tea catechin extracts for the prevention of influenza infection in elderly nursing home residents: a prospective clinical study. J Altern Complement Med 2006, 12**:**669-672.

116. Levy R, Saikovsky R, Schmidt E, Khokhlov A, Burnett BP: Efficacy of flavocoxid compared with naproxen in subjects with osteoarthritis of the knee: a randomized, double-blind pilot study. Nutr Res 2009, 29(5):298-304.

117. Levy RM, Khokhlov A, Kopenkin S, Bart B, Ermolova T, Kantemirova R, Mazurov V, Bell M, Caldron P. Pillai L, Burnett BP: Efficacy and safety of flavocoxid, a novel therapeutic, compared with naproxen in subjects with osteoarthritis of the knee. Adv Ther 2010a, 27(10):731-42.

118. Levy RM, Khokhlov A, Kopenkin S, Bart B, Ermolova T, Kantemirova R, Mazurov V, Bell M, Caldron P. Pillai L, Burnett BP: Efficacy and safety of flavocoxid compared with naproxen in subjects with osteoarthritis of the knee—a subset analysis. Adv Ther 2010b, 27(12):953-62.

119. Fiorani M, Accorsi A, Cantoni O: Human red blood cells as a natural flavonoid reservoir. Free Radic Res 2003, 37(12):1331-8.

120. LIMBREL® Post-Marketing Surveillance (2004 through March 2012), availavble at http://www.limbrel.com/downloads/post_mkt_surv.pdf

121. Sanchez-Matienzo D, Arana A, Castellsague J, Perez-Gutthann S: Hepatic disorders in patients treated with COX-2 selective inhibitors or nonselective NSAIDs: a case/noncase analysis of spontaneous reports. Clin Ther 2006, 28(8):1123-32.

122. Chalasani N, Vuppalanchi R, Navarro V, Fontana R, Bonkovsky H, Barnhart H, Kleiner DE, Hoofnagle JH: Acute liver injury due to flavocoxid (Limbrel), a medical food for osteoarthritis: A case series. Ann Intern Med 2012, 156(12):857-60.

123. Galan MV, Gordon SC, Silverman AL: Celebrex induced cholestatic hepatitis. Ann Intern Med 2001, 134(3):254.

124. El Hajj II, Malik SM, Alwakeel HR, Shaikh OS, Sasatomi E, Kandil HM: Celebrex induced cholestatic liver failure requiring orthotopic liver transplantation. World J Gastroenterol. 2009, 15(31):3937-9.

125. Youssef JG, Tomic R: Limbrel (flavocoxid) as cause of hypersensitivity pneumonitis. Chest 2010, 138(suppl 4):79A.

126. Levy RM: Flavocoxid and hypersensitivity pneumonitis. Chest 2011, 140(3):827-8.

127. Pillai L, Levy RM, Burnett BP: Results of GOAL, an open-label, post-marketing study in rheumatological practices of the efficacy, safety and gastroprotection of flavocoxid, a novel anti-inflammatory therapy. Curr Med Res Opin 2010, 26:1055-63.

128. Phase IV oral diclofenac study
http://www.emea.europa.eu/humandocs/PDFs/EPAR/onsenal/370303en6.pdf

129. Moore RA, Derry S, Phillips CJ, McQuay HJ: Nonsteroidal anti-inflammatory drugs (NSAIDs), cyxlooxygenase-2 selective inhibitors (coxibs) and gastrointestinal harm: review of clinical trials and clinical practice. BMC Musculoskelet Disord 2006, 7:79.

130. Walton SM, Schumock GT, McLain GA: Cost analysis of flavocoxid compared to naproxen for management of mild to moderate osteoarthritis. Curr Med Res Opin 2010, 26(9):2253-61.

131. Gottlieb D, Kuritzky L: Using the medical food flavocoxid in managing osteoarthritis. J Pain Palliat Care Pharmacother 2011, 25(1):49-54.

132. Rohdewald PJ. 2005. Pycnogenol®, French maritime pine bark extract. In *Encyclopedia of Dietary Supplements*, Coates PM, Blackman MR, Cragg G, Levine M, Moss J, White J (eds). Marcel Dekker: New York, 545-553.

133. Marczylo TH, Verschoyle RD, Cooke DN, Morazzoni P, Steward WP, Gescher AJ: Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. Cancer Chemother Pharmacol 2007, 60(2):171-7.

134. Antony B, A composition to enhance the bioavailability of curcumin, 44. WO2006129323, Arjuna Natural Extracts Ltd; 2006.

135. Lalithakumari K, Krishnaraju AV, Sengupta K, Subbaraju GV, Chatterjee A: Safety and Toxicological Evaluation of a Novel, Standardized 3-O-Acetyl-11-keto-beta-Boswellic Acid (AKBA)-Enriched Boswellia serrata Extract (5-Loxin(R)). Toxicol Mech Methods 2006, 16(4):199-226.

136. Thiers MH: Unsaponifiable constituents of avocado and soya oils. Treatment of certain forms of arthralgia. J Med Lyon 1972, 53(222):195-8.

137. Cisár P, Jány R, Waczulíková I, Sumegová K, Muchová J, Vojtassák J, Duraćková Z, Lisý M, Rohdewald P: Effect of pine bark extract (Pycnogenol) on symptoms of knee osteoarthritis. Phytother Res 2008,2 2(8):1087-92.

138. Belcaro G, Cesarone MR, Errichi S, Zulli C, Errichi BM, Vinciguerra G, Ledda A, Di Renzo A, Stuard S, Dugall M, Pellegrini L, Errichi S, Gizzi G, Ippolito E, Ricci A, Cacchio M, Cipollone G, Ruffini I, Fano F, Hosoi M, Rohdewald P: Treatment of osteoarthritis with Pycnogenol. The SVOS (San Valentino Osteo-arthrosis Study). Evaluation of signs, symptoms, physical performance and vascular aspects. Phytother Res 2008, 22(4):518-23.

139. Belcaro G, Cesarone MR, Dugall M, Pellegrini L, Ledda A, Grossi MG, Togni S, Appendino G: Product-evaluation registry of Meriva®, a curcumin-phosphatidylcholine complex, for the complementary management of osteoarthritis. Panminerva Med 2010, 52(2 Suppl 1):55-62.

140. Belcaro G, Cesarone MR, Dugall M, Pellegrini L, Ledda A, Grossi MG, Togni S, Appendino G: Efficacy and safety of Meriva®, a curcumin-phosphatidylcholine complex, during extended administration in osteoarthritis patients. Altern Med Rev 2010, 15(4):337-44.

141. Antony B, Kizhakkedath R, Benny M, Kuruvilla B: Clinical evaluation of a herbal formulation, Rhulief™, in the management of knee osteoarthritis. Osteoarthritis and Cartilage. 2011, 19(Suppl 1):S145-S146.

142. Sengupta K, Alluri KV, Satish AR, Mishra S, Golakoti T, Sarma KV, Dey D, Raychaudhuri SP: A double blind, randomized, placebo controlled study of the efficacy and safety of 5-Loxin for treatment of osteoarthritis of the knee. Arthritis Res Ther 2008, 10(4):R85.

143. Lequesne MG, Mery C, Samson M, Gerard P: Indexes of severity for osteoarthritis of the hip and knee. Validation--value in comparison with other assessment tests. Scand J Rheumatol Suppl 1987, 65:85-9.

144. Sengupta K, Krishnaraju AV, Vishal AA, Mishra A, Trimurtulu G, Sarma KVS, Raychaudhuri SK, Raychaudhuri SP: Comparative efficacy and tolerability of 5-Loxin[®] and Aflapin[®] against osteoarthritis of the knee: A double blind, randomized, placebo controlled clinical study. Int J Med Sci 2010, 7(6):366-377.

145. Blotman F, Maheu E, Wulwik A, Caspard H, Lopez A: Efficacy and safety of avocado/soybean unsaponifiables in the treatment of symptomatic osteoarthritis of the knee and hip. A prospective, multicenter, three-month, randomized, double-blind, placebo-controlled trial. Rev Rhum Engl Ed 1997, 64:825-834.

146. Appelboom T, Schuermans J, Verbruggen G, Henrotin Y, Reginster JY: Symptoms modifying effect of avocado/soybean unsaponifiables (ASU) in knee osteoarthritis. A double blind, prospective, placebo-controlled study. Scand J Rheumatol 2001, 30:242-247.

147. Maheu E, Mazières B, Valat JP, Loyau G, Le Loët X, Bourgeois P, Grouin JM, Rozenberg S: Symptomatic efficacy of avocado/soybean unsaponifiables in the treatment of osteoarthritis of the knee and hip: A prospective, randomized, double-blind, placebo-

controlled, multicenter clinical trial with a six-month treatment period and a two-month followup demonstrating a persistent effect. Arthritis Rheum 1998, 41:81-91.

148. Uebelhart D, Malaise M, Marcolongo R, de Vathaire F, Piperno M, Mailleux E, Fioravanti A, Matoso L, Vignon E: Intermittent treatment of knee osteoarthritis with oral chondroitin sulfate: A one-year, randomized, double-blind, multicenter study versus placebo. Osteoarthritis Cartilage 2004, 12:269-276.

149. Kahan A, Uebelhart D, De Vathaire F, Delmas PD, Reginster JY: Long-term effects of chondroitin sulfate on knee osteoarthritis: The study on osteoarthritis progression prevention, a two-year, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2009, 60:524-533.

150. Michel BA, Stucki G, Frey D, De Vathaire F, Vignon E, Bruehlmann P, Uebelhart D: Chondroitins 4 and 6 sulfate in osteoarthritis of the knee: A randomized, controlled trial. Arthritis Rheum 2005, 52:779-786.

151. Schneider H, Maheu E, Cucherat M: Symptom-modifying effect of chondroitin sulfate in knee osteoarthritis: a meta-analysis of randomized placebo-controlled trials performed with Structum(®). Open Rheumatol J 2012, 6:183-189.

152. Reichenbach S, Sterchi R, Scherer M, Trelle S, Bürgi E, Bürgi U, Dieppe PA, Jüni P: Meta-analysis: Chondroitin for Osteoarthritis of the Knee or Hip. Ann Intern Med 2007, 146:580-590.

153. Simon Wandel S, Jüni P, Tendal B, Nüesch E, Villiger PM, Welton NJ, Reichenbach S, Trelle S: Effects of glucosamine, chondroitin, or placebo in patients with osteoarthritis of hip or knee: network meta-analysis. BMJ 2010,341:c4675.