Limbrel Clinical & Scientific Abstracts Index

Note: Titles are highlighted for more important articles

I. Flavocoxid/Ingredients

1. Evaluation and Comparison of the Safety and Efficacy of UP446 and Celecoxib for the Management of Pain in Patients with Osteoarthritis

Sampalis, John S. PhD; Aurelian, Cristache M.D; and Remer, Zev M.D.

Confidential - Pending publication

Objectives: a) To evaluate the effectiveness of UP446, a dual inhibitor of cyclooxygenase and lipoxygenase, compared to placebo and active control; b) To evaluate the safety of UP446 for human consumption. UP446

Methods:This was a randomized, double blind, placebo controlled study. Eligible patients with evidence of measurable symptoms of mild to moderate osteoarthritis requiring the use of acetaminophen, anti-inflammatory agents or opioid analgesics were randomly assigned to one of four daily treatment groups: UP446 250mg, UP446 500mg, celecoxib 200mg or placebo. Patients were submitted to scheduled clinical and biochemical evaluations and responded to the Western Ontario and McMaster (WOMAC) University Osteoarthritis index and MOS SF-36 Quality of Life questionnaires.

Results: A total of 60 patients were enrolled, 22 (36.7%) men and 38 (63.3%) women with a mean age 57.6 years. UP446 was shown to be significantly effective for the management of osteoarthritis symptoms after 90 days of treatment even though effect was observed within 30 days and 60 days. UP446 was also shown to be more effective than both celecoxib and placebo. Safety analysis showed that UP446 could be considered safe for human consumption at the recommended dosage.

Conclusions: The results of the present study suggest that UP446 may be effective and significantly more effective than celecoxib for the management of the clinical course of osteoarthritis. The evaluation of safety parameters indicates that UP446 can be considered safe for human consumption.

2. Safety of UP446, a Naturally Derived, Dual Cyclooxygenase and Lipoxygenase Inhibitor

Silva, Stacia; Jia, Qi; Mesches, Michael H.; and Burnett, Bruce P.

Confidential - Pending publication

UP446, an herbal medication, has demonstrated efficacy in relief of joint pain in animals and humans. The compound derived from two plants, *Scutellaria baicalensis* and *Acacia catechu*, was found by screening over 1200 plants for cyclooxygenase (COX) and 5-lipoxygenase (LOX) dual inhibition. The active ingredients in the extracts are free-B-ring flavonoids and flavans common to diets in Asia, Australia, and Africa. Cell toxicity tests showed that UP446 was less cytotoxic than indomethacin and celecoxib in THP-1 monocytes. To assess the potential risks to humans, UP446 was tested in 2-wk acute and 13-wk subchronic toxicity studies using ICR mice. Fischer 344 rats were also tested in a 9-wk study using a dose equivalent to an effective dose in humans since rats are known to be more sensitive to non-steroidal anti-inflammatory drugs (NSAIDS) compared to mice at clinically relevant doses. Clinical signs, body weights, food and water consumption, hematology, serum biochemistry, gross findings, and histopathology in organs known to be affected by NSAIDs (stomach, liver, and kidney) were examined using non-dosed animals as controls. The results showed no abnormalities compared to the controls in any of the assessed parameters. AMES and cytochrome P450 analysis of UP446 demonstrated no specific mutagenicity or significant potential drug interactions. Based on these findings, UP466 possesses a safety profile that makes it acceptable for testing in human subjects. In particular, the dual inhibition of COX and LOX may make it more tolerable and reduce the incidence of gastrointestinal ulcerations compared to traditional NSAIDs or selective COX inhibitors.

3. Scutellariae Rudux: 7. Anti-arthritic and anti-inflammatory actions of methanolic extract and flavonoid components from Scutellariae Radix
Kubo, M.; Matsuda, H.; Tanaka, M.; Kimura, Y.; Okuda, H.; Higashino, M.; Tani, T.; Namba, K.; and Arichi, S.
Chemical and Pharmaceutical Bulletin (Tokyo). 1984;.32: 2724-2729

A 70% methanol extract of Scutellariae Radix, the dried root of Scutellaria baicalensis and its main flavonoid components, baicalin, baicalein and wogonin, were screened in comparison with 3 standard anti-inflammatory agents, phenylbutazone, indomethacin and dexamethasone, for activity in various experimental models of inflammation. All of the test substances inhibited an increase in vascular permeability in mice induced by acetic acid and reduced acute paw edema in rats induced by compound 48/80 (formaldehyde condensation product of 4-methoxy-N-methylbenzenethamine). They also suppressed the secondary lesion in developing adjuvant-induced arthritis in rats. Since these substances from Scutellariae Radix were effective in both the acute and chronic phases of inflammation, the crude drug Scutellariae Radix can be considered as having anti-inflammatory activity.

4. Anti-inflammatory properties and inhibition of leukotriene C4 biosynthesis in vitro by flavonoid baicalein from Scutellaria baicalensis georgy roots.
Butenko, IG; Gladtchenko, SV; and Galushko, SV.
Agents Actions. 1993; 39 Spec No:C49-51.

Anti-inflammatory activity of baicalein (5,6,7-trioxyflavone-7-O-beta-D-glucuronide) was greater in the chronic inflammation model (rat adjuvant arthritis, ED50 = 120.6 mg/kg) than observed in the rat carrageenan-induced paw edema, ED50 > or = 200.0 mg/kg. A comparative study of the 5-lipoxygenase (5-LO) inhibitory activity of baicalein, BW 755 C, and hydroxamic acid arachidonate on leukotriene C4 (LTC4) biosynthesis by rat resident peritoneal macrophages stimulated with calcium ionophore (A 23186) showed that these drugs significantly inhibited LTC4 production, IC50: 9.5, 41.8, and 2.8 microM, respectively. This finding suggests that inhibition of the 5-LO pathway of arachidonic acid metabolism may be one of the mechanisms of baicalein's anti-inflammatory activity.

5. Study on the anti-inflammatory mechanism of baicalin
YanNing, Hou; XiuYuan; Zhu; and GuiFang, Cheng
Acta Pharmaceutica Sinica. 2000; 35(3): 161-164 **Published in Chinese only**

The effect of baicalein on leukocyte functions was investigated to elucidate the mechanism of its anti-inflammatory action. Using rat peritoneal leukocytes, the levels of leukotrienes B4 and C4 (LTB4, LTC4), Ca2+ and cAMP were determined using HPLC, Fura-2/AM and RIA. Baicalein inhibited the biosynthesis of LTB4 and LTC4 (IC50 values of 0.48 and 3.15 micro M, respectively). Baicalein (1-100 micro M) had no effect on cytoplasmic Ca concentration of rat polymorphonuclear leukocytes (PMNL). However, baicalein at 10 and 100 micro M inhibited the increase in Ca2+ level induced by fMLP by 29 and 40%, respectively. Baicalein (1, 10 and 100 micro M) increased cAMP levels in rat PMNL by 74, 103 and 138%, respectively. The effects of baicalein on leukocyte function may provide the basis for its anti-inflammatory actions.

6. Mechanisms in mediating the anti-inflammatory effects of baicalin and baicalein in human leukocytes.
Shen, Y. C.; Chiou, W. F.; Chou, Y. C.; and Chen, C. F.
Eur J Pharmacol. 2003; 465(1-2): 171-81

To evaluate the possible mechanisms responsible for the anti-inflammatory effects of baicalin or baicalein, phorbol-12-myristate-13-acetate (PMA)- or N-formyl-methionyl-leucyl-phenylalanine (fMLP)-activated inflammatory responses of peripheral human leukocytes were studied. Both baicalin and baicalein diminished fMLP- or PMA-induced reactive oxygen intermediates production in neutrophils or monocytes. Neither baicalin nor baicalein prevented the protein kinase C (PKC)-dependent assembly of the NADPH oxidase. Conversely, myeloperoxidase (MPO) activity was inhibited by baicalin or baicalein. fMLP-induced activation of leukocytes, as reflected by increased surface expression of Mac-1 (CD11b/CD18) and Mac-1-dependent neutrophil adhesion, were also inhibited by baicalin or baicalein. Furthermore, baicalein, but not baicalin, impeded fMLP- or AlF(4)(-)-

induced Ca(2+) influx. We conclude that impairment of reactive oxygen intermediates production, through scavenging reactive oxygen intermediates by baicalin, or antagonizing ligand-initiated Ca(2+) influx by baicalein, accounts for the inhibition of Mac-1-dependent leukocyte adhesion that confers the anti-inflammatory activity of baicalin or baicalein.

7. The anti-inflammatory and analgesic effects of baicalin in carrageenan-evoked thermal hyperalgesia.
Chou, T. C.; Chang, L. P.; Li, C. Y.; Wong, C. S.; and Yang, S. P.
Anesth Analg. 2003; 97: 1724-9

We tested baicalin for its anti-inflammatory and analgesic effects (and the mechanisms) in a rat model of carrageenan-evoked thermal hyperalgesia. Pre- or posttreatment with baicalin (10, 30, or 100 mg/kg intraperitoneally) caused a significant analgesic effect with a similar effect of dose-matched ibuprofen. Furthermore, baicalin dose-dependently attenuated tumor necrosis factor-alpha (from 3510 +/- 150 pg/mL to 2860 +/- 148 pg/mL to 1480 +/- 210 pg/mL), interleukin (IL)-1beta (from 3210 +/- 210 pg/mL to 2200 +/- 140 pg/mL to 750 +/- 95 pg/mL), and IL-6 (from 58.5 +/- 9.8 pg/mL to 38.5 +/- 9.0 to 21.0 +/- 8.1 ng/mL) formation but enhanced IL-10 (from 18.1 +/- 2.5 pg/mL to 36.1 +/- 5.5 pg/mL to 71.2 +/- 9.5 pg/mL) production in paw exudates at 4 h after carrageenan injection. Prostaglandin E(2) (PGE(2)) and nitrate formation in the carrageenan-injected paws were dose-dependently inhibited by baicalin (10-100 mg/kg intraperitoneally) (PGE(2): from 15.9 +/- 2.1 ng/mL to 12.1 +/- 1.6 ng/mL to 6.2 +/- 1.8 ng/mL; nitrate: from 39.8 +/- 4.8 microM to 27.5 +/- 3.0 microM to 17.2 +/- 1.6 microM) at 4 h butnot at 1.5 h after carrageenan injection. Increased myeloperoxidase activity in carrageenan-injected paws was also dose-dependently reduced by baicalin These findings suggest that the anti-inflammatory and analgesic mechanisms of baicalin may be associated with the inhibition of critical inflammatory mediators, including nitric oxide, PGE(2), and proinflammatory cytokines, accompanied by an increase in IL-10 production, as well as neutrophil infiltration at sites of inflammation. IMPLICATIONS: Our results showed that baicalin possesses an analgesic effect in carrageenan-evoked thermal hyperalgesia. The possible mechanisms of action of baicalin may be associated with the inhibition of proinflammatory mediator overproduction, including cytokines, nitric oxide, and prostaglandin E(2), as well as neutrophil infiltration. This implies that baicalin may be a potential therapeutic analgesic for inflammatory pain.

8. Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expressions induced by nitric oxide synthase inhibitors and lipopolysaccharide.
Chen, YC; Shen, SC; Chen, LG; Lee, TJ; and Yang, LL.
Biochem Pharmacol. 2001 Jun 1; 61(11): 1417-27.

We previously reported that oroxylin A, a polyphenolic compound, was a potent inhibitor of lipopolysaccharide (LPS)-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). In the present study, three oroxylin A structurally related polyphenols isolated from the Chinese herb Huang Qui, namely baicalin, baicalein, and wogonin, were examined for their effects on LPS-induced nitric oxide (NO) production and iNOS and COX-2 gene expressions in RAW 264.7 macrophages. The results indicated that these three polyphenolic compounds inhibited LPS-induced NO production in a concentration-dependent manner without a notable cytotoxic effect on these cells. The decrease in NO production was in parallel with the inhibition by these polyphenolic compounds of LPS-induced iNOS gene expression. However, these three compounds did not directly affect iNOS enzyme activity. In addition, wogonin, but not baicalin or baicalein, inhibited LPS-induced prostaglandin E2 (PGE2) production and COX-2 gene expression without affecting COX-2 enzyme activity. Furthermore, N-nitro-L-arginine (NLA) and N-nitro-L-arginine methyl ester (L-NAME) pretreatment enhanced LPS-induced iNOS (but not COX-2) protein expression, which was inhibited by these three polyphenolic compounds. Wogonin, but not baicalin or baicalein, similarly inhibited PGE2 production and COX-2 protein expression in NLA/LPS or L-NAME/LPS-co-treated RAW 264.7 cells. These results indicated that co-treatment with NOS inhibitors and polyphenolic compounds such as wogonin effectively blocks acute production of NO and, at the same time, inhibits expression of iNOS and COX-2 genes.

9. Baicalein attenuates oxidant stress in cardiomyocytes.
Shao, ZH; Vanden Hoek, TL; Qin, Y; Becker, LB; Schumacker, PT; L,i CQ; Dey, L; Barth, E; Halpern, H; Rosen, GM; and Yuan, CS
Am J Physiol Heart Circ Physiol. 2002; 282(3): H999-H1006.

Flavonoids within Scutellaria baicalensis may be potent antioxidants on the basis of our studies of S. baicalensis extract. To further this work, we studied the antioxidative effects of baicalein, a flavonoid component of S. baicalensis, in a chick cardiomyocyte model of reactive oxygen species (ROS) generation during hypoxia, simulated ischemia-reperfusion, or mitochondrial complex III inhibition with antimycin A. Oxidant stress was measured by oxidation of the intracellular probes 2',7'-dichlorofluorescin diacetate and dihydroethidium. Viability was assessed by propidium iodide uptake. Baicalein attenuated oxidant stress during all conditions studied and acted within minutes of treatment. For example, baicalein given only at reperfusion dose dependently attenuated the ROS burst at 5 min after 1 h of simulated ischemia. It also decreased subsequent cell death at 3 h of reperfusion from 52.3 +/- 2.5% in untreated cells to 29.4 +/- 3.0% (with return of contractions; P < 0.001). In vitro studies using electron paramagnetic resonance spectroscopy with the spin trap 5-methoxycarbonyl-5-methyl-1-pyrroline-N-oxide revealed that baicalein scavenges superoxide but does not mimic the effects of superoxide dismutase. We conclude that baicalein can scavenge ROS generation in cardiomyocytes and that it protects against cell death in an ischemia-reperfusion model when given only at reperfusion.

10. Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin.
Shieh, DE; Liu, LT; and Lin, CC.
Anticancer Res. 2000; 20(5A): 2861-5.

Xanthine oxidase inhibitors are known to be therapeutically useful for the treatment of hepatitis and brain tumor. Baicalein, baicalin and wogonin, isolated from Scutellaria rivularis, have been reported to exhibit a strong activity on xanthine oxidase inhibition. In this study, their antioxidant activity was evaluated by modified xanthine oxidase inhibition and cytochrome c reduced methods. The results showed that the order of activity on xanthine oxidase inhibition was baicalein > wogonin > baicalin, IC50 = 3.12, 157.38 and 215.19 microM, respectively, whereas the activity on cytochrome c reduction was baicalin > wogonin > baicalein (IC50 = 224.12, 300.10 and 370.33 microM, respectively). In another study, an electron spin resonance (ESR) technique was used to further confirm the direct free radical scavenging activity. Both baicalein and baicalin demonstrated a strong activity on eliminating the superoxide radical (.O2-) (baicalein: 7.31 x 10(4) u/g; baicalin: 1.19 x 10(5) u/g). The IC50 of baicalein was 2.8 fold higher than that of baicalin. However they had no significant effect on scavenging hydroxyl radical (.OH). The present results demonstrated that baicalein and baicalin posed a different pathological pathway. The antioxidant function of baicalin was mainly based on scavenging superoxide radical whilst baicalein was a good xanthine oxidase inhibitor

11. Catechins from green tea (Camellia sinensis) inhibit bovine and human cartilage proteoglycan and type II collagen degradation in vitro.
Adcocks, C; Collin, P; and Buttle, DJ.
J Nutr. 2002; 132(3): 341-6.

Polyphenolic compounds from green tea have been shown to reduce inflammation in a murine model of inflammatory arthritis, but no studies have been undertaken to investigate whether these compounds are protective to joint tissues. We therefore investigated the effects of catechins found in green tea on cartilage extracellular matrix components using in vitro model systems. Bovine nasal and metacarpophalangeal cartilage as well as human nondiseased, osteoarthritic and rheumatoid cartilage were cultured with and without reagents known to accelerate cartilage matrix breakdown. Individual catechins were added to the cultures and the amount of released proteoglycan and type II collagen was measured by metachromatic assay and inhibition ELISA, respectively. Possible nonspecific or toxic effects of the catechins were assessed by lactate output and proteoglycan synthesis. Catechins, particularly those containing a gallate ester, were effective at micromolar concentrations at inhibiting proteoglycan and type II collagen breakdown. No toxic effects of the catechins were evident. We conclude that some green tea catechins are chondroprotective and that consumption of green tea may be prophylactic for arthritis and may benefit the arthritis patient by reducing inflammation and slowing cartilage breakdown. Further studies will be required to determine whether these compounds access the joint space in sufficient concentration and in a form capable of providing efficacy in vivo.

12. Green tea polyphenol epigallocatechin-3-gallate inhibits the IL-1 beta-induced activity and expression of cyclooxygenase-2 and nitric oxide synthase-2 in human chondrocytes.
Ahmed S; Rahman, A; Hasnain, A; Lalonde, M; Goldberg, VM; and Haqqi TM.
Free Radic Biol Med. 2002; 33(8): 1097-105.

We have previously shown that green tea polyphenols inhibit the onset and severity of collagen II-induced arthritis in mice. In the present study, we report the pharmacological effects of green tea polyphenol epigallocatechin-3-gallate (EGCG), on interleukin-1 beta (IL-1 beta)-induced expression and activity of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in human chondrocytes derived from osteoarthritis (OA) cartilage. Stimulation of human chondrocytes with IL-1 beta (5 ng/ml) for 24 h resulted in significantly enhanced production of nitric oxide (NO) and prostaglandin E(2) (PGE(2)) when compared to untreated controls ($p < .001$). Pretreament of human chondrocytes with EGCG showed a dose-dependent inhibition in the production of NO and PGE(2) by 48% and 24%, respectively, and correlated with the inhibition of iNOS and COX-2 activities ($p < .005$). In addition, IL-1 beta-induced expression of iNOS and COX-2 was also markedly inhibited in human chondrocytes pretreated with EGCG ($p < .001$). Parallel to these findings, EGCG also inhibited the IL-1 beta-induced LDH release in chondrocytes cultures. Overall, the study suggests that EGCG affords protection against IL-1 beta-induced production of catabolic mediators NO and PGE(2) in human chondrocytes by regulating the expression and catalytic activity of their respective enzymes. Furthermore, our results also indicate that ECGC may be of potential therapeutic value for inhibiting cartilage resorption in arthritic joints.

13. The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines.
Li, BQ; Fu, T; Gong, WH; Dunlop, N; Kung, H; Yan, Y; Kang, J; and Wang, JM.
Immunopharmacology. 2000; 49(3): 295-306

Baicalin (BA) is a flavonoid compound purified from the medicinal plant Scutellaria baicalensis Georgi and has been reported to possess anti-inflammatory and anti-viral activities. In order to elucidate the mechanism(s) of action of BA, we tested whether BA could interfere with chemokines or chemokine receptors, which are critical mediators of inflammation and infection. We observed that BA inhibited the binding of a number of chemokines to human leukocytes or cells transfected to express specific chemokine receptors. This was associated with a reduced capacity of the chemokines to induce cell migration. Co-injection of BA with CXC chemokine interleukin-8 (IL-8) into rat skin significantly inhibited IL-8 elicited neutrophil infiltration. BA did not directly compete with chemokines for binding to receptors, but rather acted through its selective binding to chemokine ligands. This conclusion was supported by the fact that BA cross-linked to oxime resin bound chemokines of the CXC (stromal cell-derived factor (SDF)-1alpha, IL-8), CC (macrophage inflammatory protein (MIP)-1beta, monocyte chemotactic protein (MCP)-2), and C (lymphotactin (Ltn)) subfamilies. BA did not interact with CX3C chemokine fractalkine/neurotactin or other cytokines, such as TNF-alpha and IFN-gamma, indicating that its action is selective. These results suggest that one possible anti-inflammatory mechanism of BA is to bind a variety of chemokines and limit their biological function.

14. The anti-inflammatory activity of Scutellaria rivularis extracts and its active components, baicalin, baicalein and wogonin.
Lin, C. C. and Shieh, D. E.
Am J Chin Med. 1996; 24: 31-6

Five extracts (n-hexane, chloroform, ethyl acetate, n-butanol and water) of Scutellaria rivularis Benth. were evaluated for their anti-inflammatory activity against carrageenan-induced paw edema in rats and compared with indomethacin. The result indicated that chloroform extract proved to be the most effective in all of the extracts. Consequently, three major components (baicalin, baicalein and wogonin) of the chloroform extract were further tested for their anti-inflammatory activity using the same model. It was found that baicalin exhibits the greatest inhibition activity against carrageenan-induced rat paw edema.

15. The flavonoid baicalin inhibits superantigen-induced inflammatory cytokines and chemokines.
Krakauer, T.; Li, B. Q.; and Young, H. A.
FEBS Lett. 2001; 500(1-2): 52-5

Excessive release of proinflammatory cytokines mediates the toxic effect of superantigenic staphylococcal exotoxins (SE). Baicalin, a flavone isolated from the Chinese herb Scutellaria baicalensis Georgi and used in China to treat infectious diseases, inhibited SE-stimulated T-cell proliferation (by 98%) and production of interleukin 1beta, interleukin 6, tumor necrosis factor, interferon gamma, monocyte chemotactic protein 1, macrophage inflammatory protein (MIP)-1alpha, and MIP-1beta mRNA and protein by human peripheral blood mononuclear cells. These data suggest that baicalin may be therapeutically useful for mitigating the pathogenic effects of SE by inhibiting the signaling pathways activated by superantigens.

16. Effects of baicalein isolated from Scutellaria baicalensis on interleukin 1beta- and tumor necrosis factor alpha-induced adhesion molecule expression in cultured human umbilical vein endothelial
Kimura, Y.; Matsushita, N.; and Okuda, H.
Journal of Ethnopharmacology (Ireland). 1997; 57: 63-67

To investigate the mechanism of anti-inflammatory effects of 9 flavonoids, including baicalein, isolated from Scutellariae radix, varying concentrations of the flavonoids underwent analysis using interleukin 1beta- and tumor necrosis factor-alpha-induced adhesion molecule expression in cultured human umbilical vein endothelial cells. Baicalein inhibited interleukin 1beta- and tumor necrosis factor-alpha-induced adhesion molecule expressions in a dose dependent manner.

17. Antagonism of saikosaponin-induced prostaglandin E2 release by baicalein in C6 rat glioma cells.
Kyo, R.; Nakahata, N.; Kodama, Y.; Nakai, Y.; Kubo, M.; and Ohizumi, Y.
Biological & Pharmaceutical Bulletin. 1999; 22: 1385-1387

There are several Kampo medicines containing both Bupleuri Radix (Bupleurum falcatum) and Scutellariae Radix (Scutellaria baicalensis) which are used to treat inflammation Saikosaponins are derived from Bupleuri Radix, and baicalein is from Scutellariae Radix. The present study was undertaken to investigate the pharmacological interaction of saikosaponin b1 and baicalein in prostaglandin E2 (PGE2) release from C6 rat glioma cells in vitro. Saikosaponins a, b1 and d potently stimulated PGE2 release, while saikosaponins b2 and c moderately stimulated PGE2 release. Saikosaponin b1 caused an irreversible elevation of intracellular Ca2+ concentration, which was eliminated by removing extracellular Ca2+. Baicalein inhibited saikosaponin b1-induced PGE2 release in a concentration-dependent manner. These results suggest that saikosaponins are activators of PGE2 release, and baicalein is one of the functional inhibitors of PGE2 release by saikosaponins.

18. Experimental study of the anti-inflammatory function of total flavonoids of leaves and stems of Scutellaria baicalensis - Published in Chinese only
Zhou XiaoXia et al
Chinese Journal of Information on Traditional Chinese Medicine. 2000; 7: 27-

In mice the administration of total flavonoids extracted from S. biacalensis reduced inflammation more effectively than hydrocortisone when given at 100 mg/kg. It was less effective than hydrocortisone when administered at 50 mg/kg. The flavonoids caused opening of blood capillaries.

19. Extract from Scutellaria baicalensis Georgi attenuates oxidant stress in cardiomyocytes.
Yuan, Chun-Su; Attele, Anoja S.; Becker, Lance B.; Li, Chang-Qing; Schumacker, Paul T.; Shao, Zuo-Hui; Vanden Hoek, Terry L.; and Wu, Ji A.
Journal of Molecular and Cellular Cardiology. 1999; 31(10): 1885-1895.

Scutellaria baicalensis Georgi is a Chinese herbal medicine used to treat allergic and inflammatory diseases. The medicinal effects of S. biacalensis root may result, in part, from its constituent flavones reported to have antioxidant properties. Since oxidants play multiple roles in cells, we tested whether S. biacalensis could confer

protection in a cardiomyocyte of ischemia and reperfusion. The intracellular fluorescent probes 2',7'-dichlorofluorescin diacetate (DCFH-DA, sensitive to H2O2 and hydroxyl radicals) and dihydroethidium (DHE, sensitive to superoxide) were used to assess intracellular reactive oxygen species (ROS), and propidium iodide (PI) was used to assess viability in cultured embryonic cardiomyocytes. S. biacalensis extract (SbE) quickly attenuated levels of oxidants generated during transient hypoxia and during exposure to the mitochondrial site III inhibitor antimycin A, as measured by DCFH oxidation or by DHE oxidation. These attenuated oxidant levels wer e associated with improved survival and function. Cell death after ischemia/ reperfusion decreased from 47 +- 3% in untreated to 26 +- 2% in S. biacalensis treated cells (P<0.001). After antimycin A exposure, S. biacalensis decreased cell death from 49 +- 6% in untreated to 23 +- 4% in treated cells. Return of contraction occurred in S. baicalensis-treated cells but was not observed in control cells. Other in vitro studies revealed that baicalein, a major flavone component of SbE can directly scavenge superoxide, hydrogen peroxide, and hydroxyl radicals. Collectively, these findings indicate that SbE and its constituent flavones such as baicalein can attenuate oxidant stress and protect cells from lethal oxidant damage in an ischemia-reperfusion model.

20. The Bailal skullcap (Scutellaria baicalensis Georgi)--a potential source of new drugs
Martin, J. and Dusek, J.
Ceska Slov Farm. 2002; 51: 277-83 **Full article omitted – published in Czech**

The scullcap (Scutellaria baicalensis Georgi) is a medicinal plant of traditional Chinese medicine and the drug--Scutellariae radix--is, because of its antioxidant, antiviral, antibacterial, anti-inflammatory, antiallergic, and sedative properties, the subject of intensive development. This paper reports the results of pharmacological-toxicological studies of this drug and its flavonids approximately from the year 1990 till the year 2001. The results confirm the validity of traditional use and at the same time indicate that some flavonoids have more utilizable therapeutic effects. Mainly baicalein seems to be a prospective medicine for the treatment of some kinds of cancer.

21. Analysis of flavonoid contents in Scutellaria by HPLC and LC-MS. Full article omitted-Not available
Wang, Mingfu; Simon, James E.; Joshee, Nirmal; Yadav, and Anand K.
Hortscience. 2003; 38(5): 826-827

The species of Scutellaria Plants contains a lot of phtytochemicals including flavonoids, Diterpenes, Triterpenes, ligans and other phenolic compounds. The major compounds were found to be flavonoids and a few of flavonoids have been isolated from Scutellaria including baicalein, baicalin, wogonin, apigenin, luteolin etc. These compounds have a lot of pharmacological activities, especially three compounds, baicalein, baicalin and wogonin have been studied extensively and they have antioxidant activity, anti-hepatitis B virus, anti-inflammatory and anti-HIV activities. In this research, Phytochemicals from different parts (roots, leaves and stems) were extracted using aqueous alcohol solution with the aid of sonication. Then a HPLC method was developed and validated to determine the total flavonoids, the contents of baicalein, baicalein, wogonin and other flavonoids in them. The flavonoids in Scutellaria were identified by retention time of components, on-line UV spectrum, where possible, comparison will be made with authentic standards. LC-MS and LC/MS/MS methods were also developed to identify and analyze the flavonoids in Scutellaria. The structures of unknown compounds were also tentatively given based on the MS and MS/MS data.

22. Evaluation of the anti-inflammatory effect of baicalein on dextran sulfate sodium-induced colitis in mice.
Hong, T; Jin, GB; Cho, S; and Cyong, JC.
Planta Med. 2002; 68(3): 268-71.

The anti-inflammatory effect of three flavonoids from the root of Scutellaria baicalensis (baicalein, baicalin and wogonin) was evaluated in a murine model of acute experimental colitis induced by dextran sulfate sodium (DSS). Baicalein, but not baicalin or wogonin, given orally at 20 mg/kg for ten days, ameliorates all the considered inflammatory symptoms of the induced colitis, such as body weight loss, blood haemoglobin content, rectal bleeding and other histological and biochemical parameters. The effect of baicalein was similar to that of sulfasalazine, the reference drug given at 50 mg/kg.

23. Inhibition of cancer cell proliferation and prostaglandin E2 synthesis Scutellaria baicalensis
Zhang, D. Y; Wu, J.; Ye, F.; Xue, L.; Jiang, S.; Yi, J.; Zhang, W.; Wei, H.; Sung, M.; Wang, W.; and Li, X.
Cancer Res. 2003; 63 (14): 4037-43

Scutellaria baicalensis is a widely used Chinese herbal medicine that has been used historically in anti-inflammatory and anticancer therapy. The purpose of this study is to verify its anticancer activity on head and neck squamous cell carcinoma (HNSCC) in vitro and in vivo and to investigate its effect on cyclooxygenase-2 (COX-2), which converts arachidonic acid to prostaglandin E(2) (PGE(2)) and is highly expressed in HNSCC. Two human HNSCC cell lines (SCC-25 and KB) and a nontumorigenic cell line (HaCaT) were tested in vitro for growth inhibition, proliferation cell nuclear antigen expression, and COX-2 activity and expression after treatment with Scutellaria baicalensis extract. Its effects were compared with those of baicalein (a flavonoid isolated from Scutellaria baicalensis), indomethacin (a nonselective COX inhibitor), and celecoxib (a selective COX-2 inhibitor). Four nude mice with s.c. inoculation of KB cells were tested for its anticancer activity in vivo by oral administration of Scutellaria baicalensis at a dose of 1.5 mg/mouse (75 mg/kg), five times/week for 7 weeks. Scutellaria baicalensis and other agents demonstrated a strong growth inhibition in both tested human HNSCC cell lines. No growth inhibition of HaCaT cells was observed with Scutellaria baicalensis. The IC(50)s were 150 micro g/ml for Scutellaria baicalensis, 25 micro M for celecoxib, and 75 micro M for baicalein and indomethacin. Scutellaria baicalensis, as well as celecoxib and indomethacin, but not baicalein, suppressed proliferation cell nuclear antigen expression and PGE(2) synthesis in both cell types. Scutellaria baicalensis inhibited COX-2 expression, whereas celecoxib inhibited COX-2 activity directly. A 66% reduction in tumor mass was observed in the nude mice. Scutellaria baicalensis selectively and effectively inhibits cancer cell growth in vitro and in vivo and can be an effective chemotherapeutic agent for HNSCC. Inhibition of PGE(2) synthesis via suppression of COX-2 expression may be responsible for its anticancer activity. Differences in biological effects of Scutellaria baicalensis compared with baicalein

24. Pharmacological effects of methanolic extract from the root of Scutellaria baicalensis and its flavonoids on human gingival fibroblast
Chung, C. P.; Park, J. B.; and Bae, K. H.
Planta Medica . 1995; 61: 150-153

The in vitro anti-inflammatory effects and human gingival fibroblast activation of the methanolic extract of Scutellaria baicalensis and its flavonoids, baicalein, baicalin, and wogonin are reported.

25. Inhibitory effect of baicalein, a flavonoid in Scutellaria Root, on eotaxin production by human dermal fibroblasts.
Nakajima, T.; Imanishi, M.; Yamamoto, K.; Cyong JongChol; and Hirai, K.
Planta Medica. 2001; 67: 132-135

Eotaxin is an eosinophil-specific chemokine associated with the recruitment of eosinophils to sites of allergic inflammation "Saiboku-to" is a kampo herbal medicine used for the treatment of bronchial asthma in Japan. In this study, we investigated the effects of the root of Scutellaria, a major herb in Saiboku-to and its components such as baicalein and baicalin on eotaxin production by IL-4 plus TNF- alpha -stimulated human fibroblasts. The root extract markedly inhibited eotaxin production. Four major flavonoids from the roots were found to show inhibitory activity on eotaxin production at a concentration of 10 micro g/ml in the order of baicalein>oroxylin A>baicalin>skullcapflavon II. The inhibitory effect of baicalein was expressed in a dose-dependent manner, and almost 50% inhibition was observed at 1.8 micro g/ml. Furthermore, baicalein prevented human eotaxin mRNA expression in IL-4 plus TNF- alpha -stimulated human fibroblasts. These results help explain the pharmacological efficacy of Scutellaria root in the treatment of bronchial asthma since it would suppress eotaxin associated recruitment of eosinophils.

26. Anticancer activity of Scutellaria baicalensis and its potential mechanism
Ye, F; Xui, L; Yi, JZ; Zhang, WD; and Zhang, DY
Journal of Alternative and Complementary Medicine. 2002; 8(5): 567-572

Objective: Scutellaria baicalensis is a widely used Chinese herbal medicine that historically is used in anti-inflammatory and anticancer therapy. The aim of the study is to determine its ability to inhibit human cancer cells in vitro and to determine whether its anticancer activity is because of the inhibition of prostaglandin E<INF>2</INF> (PGE<INF>2</INF>) production that is derived from arachidonic acid through cyclooxygenase-2 (COX-2) pathway. Methods: Cell lines from the most common human cancers, including squamous cell carcinoma (SCC-25, KB), breast cancer (MCF-7), hepatocellular carcinoma (HepG2), prostate carcinoma (PC-3 and LNCaP), and colon cancer (KM-12 and HCT-15) were tested. The cells were treated with various concentrations of Scutellaria baicalensis (0.1-100 mg/mL) for 72 hours. Percentage of viable cells after treatment was assessed using a trypan blue dye exclusion assay and the level of PGE2 production was determined by enzyme immunoassay (EIA). Results: Scutellaria baicalensis demonstrated a strong dose-dependent growth inhibition in all cell lines. Inhibition concentration at 50% (IC<INF>50</INF>) for HepG2, MCF-7, PC-3, LNCaP, KM-12, HCT-15, KB and SCC-25 cells was 1.1, 0.9, 0.52, 0.82, 1.1, 1.5, 1.0, and 1.2 mg/mL, respectively. Three cell lines (KB, SCC-25, and HepG2) were assessed for the production of PGE2 and a high level of extracellular (KB and SCC-25) and intracellular PGE2 (HepG2) was noted. In the presence of Scutellaria baicalensis extract, there was a significant decrease of PGE2 in a dose-dependent fashion. Conclusions: Scutellaria baicalensis strongly inhibits cell growth in all cancer cell lines tested. However, prostate and breast cancer cells (PC-3, LNCaP, and MCF-7) are slightly more sensitive than other type of cancer cells. It also inhibits PGE2 production, indicating that suppression of tumor cell growth may be due to its ability to inhibit COX-2 activity. This study supports the notion of using Scutellaria baicalensis as a novel anticancer agent to treat various cancers.

II. Ingredients Class (flavonoids)

27. Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids
Ferrandiz, ML and Alcaraz, MJ.
Agents Actions. 1991; 32(3-4): 283-8.

A group of flavonoids isolated from medicinal plants and which are selective inhibitors of lipoxygenase activity in vitro: sideritoflavone, cirsiliol, hypolaetin-8-O-beta-D-glucoside, hypolaetin, oroxindin, quercetagetin-7-O-beta-D-glucoside, gossypin, hibifolin and gossypetin, besides leucocyanidol, have been studied for their effects on acute responses induced by carrageenin in mice. The oral administration of flavonoids to mice inhibited dose-dependently the development of paw oedema at 1, 3 and 5 h after carrageenin injection. A similar administration of flavonoids induced a dose-dependent inhibition of leukocyte accumulation in inflammatory exudates following intraperitoneal injection of carrageenin into mice. Some of the flavonoids exhibited a potency against leukocyte infiltration similar to that seen for inhibition of carrageenin oedema at 3 h of induction. In agreement with data reported in rats, indomethacin was much more effective on inhibition of prostaglandin E2 (PGE2) formation than on leukocyte infiltration in mice. The selectivity of flavonoids towards lipoxygenase is not retained in vivo since they behave as dual inhibitors of PGE2 and leukotriene B4 (LTB4) formation in peritoneal exudates. Our data support the inhibition of arachidonic acid metabolism as one of the mechanisms by which flavonoids exert their anti-inflammatory effects.

28. Biological properties of flavonoids pertaining to inflammation.
Manthey, JA.
Microcirculation. 2000; 7(6 Pt 2): S29-34.

Chronic venous insufficiency (CVI) is accompanied by a marked inflammatory response that is thought to contribute to the development and progression of the disorder. While compression therapy has long been considered the standard treatment for CVI, recent studies suggest that treatment with flavonoids may also be beneficial. The purpose of this review is to summarize how plant flavonoids attenuate inflammation and the immune response through their inhibition of important regulatory enzymes. Certain flavonoids are potent inhibitors of the production of prostaglandins, a group of powerful proinflammatory signaling molecules. Studies

have shown that this effect is due to flavonoid inhibition of key enzymes involved in prostaglandin biosynthesis (i.e., lipoxygenase, phospholipase, and cyclooxygenase). Flavonoids also inhibit phosphodiesterases involved in cell activation. Much of this effect is upon the biosynthesis of protein cytokines that mediate adhesion of circulating leukocytes to sites of injury. The protein kinases are another class of regulatory enzymes affected by flavonoids. The inhibition of kinases is due to the competitive binding of flavonoids with ATP at catalytic sites on the enzymes. These modes of inhibition provide the mechanisms by which flavonoids inhibit the inflammation response and suggest that this class of molecules may be effective in the treatment of CVI.

29. Effect of plant flavonoids on immune and inflammatory cell function.
Middleton, E Jr.
Adv Exp Med Biol. 1998; 439: 175-82.

The flavonoids are a large group of naturally occurring phenylchromones found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Up to several hundred milligrams are consumed daily in the average Western diet. Only limited information is available on the absorption, distribution, metabolism, and excretion of these compounds in man. Some compounds are absorbed, however, and measurable plasma concentrations are achieved which could have pharmacological relevance. A variety of in vitro and in vivo experiments have shown that selected flavonoids possess antiallergic, antiinflammatory, antiviral and antioxidant activities. Moreover, acting by several different mechanisms, particular flavonoids can exert significant anticancer activity including anticarcinogenic properties and even a prodifferentiative activity, amongst other modes of action. Certain flavonoids possess potent inhibitory activity against a wide array of enzymes, but of particular note is their inhibitory effects on several enzyme systems intimately connected to cell activation processes such as protein kinase C, protein tyrosine kinases, phospholipase A2, and others. Evidence suggests that only activated cells are susceptible to the modulating effects of flavonoids, i.e. cells which are responding to a stimulus. The stimulated activities of numerous cell types, including mast cells, basophils, neutrophils, eosinophils, T & B lymphocytes, macrophages, platelets, smooth muscle, hepatocytes, and others, can be influenced by particular flavonoids. On balance, a considerable body of evidence suggests that plant flavonoids may be health-promoting, disease-preventing dietary compounds.

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

Flavonoids are nearly ubiquitous in plants and are recognized as the pigments responsible for the colors of leaves, especially in autumn. They are rich in seeds, citrus fruits, olive oil, tea, and red wine. They are low molecular weight compounds composed of a three-ring structure with various substitutions. This basic structure is shared by tocopherols (vitamin E). Flavonoids can be subdivided according to the presence of an oxy group at position 4, a double bond between carbon atoms 2 and 3, or a hydroxyl group in position 3 of the C (middle) ring. These characteristics appear to also be required for best activity, especially antioxidant and antiproliferative, in the systems studied. The particular hydroxylation pattern of the B ring of the flavonoles increases their activities, especially in inhibition of mast cell secretion. Certain plants and spices containing flavonoids have been used for thousands of years in traditional Eastern medicine. In spite of the voluminous literature available, however, Western medicine has not yet used flavonoids therapeutically, even though their safety record is exceptional. Suggestions are made where such possibilities may be worth pursuing.

31. Effects of flavonoids on immune and inflammatory cell functions.
Middleton, E Jr. And Kandaswami, C.
Biochem Pharmacol. 1992; 43(6): 1167-79.

No doubt can remain that the flavonoids have profound effects on the function of immune and inflammatory cells as determined by a large number and variety of in vitro and some in vivo observations. That these ubiquitous dietary chemicals may have significant in vivo effects on homeostasis within the immune system and on the behavior of secondary cell systems comprising the inflammatory response seems highly likely but more work is required to strengthen this hypothesis. Ample evidence indicates that selected flavonoids, depending on

structure, can affect (usually inhibit) secretory processes, mitogenesis, and cell-cell interactions including possible effects on adhesion molecule expression and function. The possible action of flavonoids on the function of cytoskeletal elements is suggested by their effects on secretory processes. Moreover, evidence indicates that certain flavonoids may affect gene expression and the elaboration and effects of cytokines and cytokine receptors. How all of these effects are mediated is not yet clear but one important mechanism may be the capacity of flavonoids to stimulate or inhibit protein phosphorylation and thereby regulate cell function. Perhaps the counterbalancing effect of cellular protein tyrosine phosphatases will also be found to be affected by flavonoids. Some flavonoid effects can certainly be attributed to their recognized antioxidant and radical scavenging properties. A potential mechanism of action that requires scrutiny, particularly in relation to enzyme inhibition, is the redox activity of appropriately configured flavonoids. Finally, in a number of cell systems it seems that resting cells are not affected significantly by flavonoids but once a cell becomes activated by a physiological stimulus a flavonoid-sensitive substance is generated and interaction of flavonoids with that substance dramatically alters the outcome of the activation process.

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

BACKGROUND: Flavonoids are effective antioxidants and may protect against several chronic diseases. OBJECTIVE: The association between flavonoid intake and risk of several chronic diseases was studied. DESIGN: The total dietary intakes of 10 054 men and women during the year preceding the baseline examination were determined with a dietary history method. Flavonoid intakes were estimated, mainly on the basis of the flavonoid concentrations in Finnish foods. The incident cases of the diseases considered were identified from different national public health registers. RESULTS: Persons with higher quercetin intakes had lower mortality from ischemic heart disease. The relative risk (RR) between the highest and lowest quartiles was 0.79 (95% CI: 0.63, 0.99: P for trend = 0.02). The incidence of cerebrovascular disease was lower at higher kaempferol (0.70; 0.56, 0.86; P = 0.003), naringenin (0.79; 0.64, 0.98; P = 0.06), and hesperetin (0.80; 0.64, 0.99; P = 0.008) intakes. Men with higher quercetin intakes had a lower lung cancer incidence (0.42; 0.25, 0.72; P = 0.001), and men with higher myricetin intakes had a lower prostate cancer risk (0.43; 0.22, 0.86; P = 0.002). Asthma incidence was lower at higher quercetin (0.76; 0.56, 1.01; P = 0.005), naringenin (0.69; 0.50, 0.94; P = 0.06), and hesperetin (0.64; 0.46, 0.88; P = 0.03) intakes. A trend toward a reduction in risk of type 2 diabetes was associated with higher quercetin (0.81; 0.64, 1.02; P = 0.07) and myricetin (0.79; 0.62, 1.00; P = 0.07) intakes. CONCLUSION: The risk of some chronic diseases may be lower at higher dietary flavonoid intakes.

33. Dietary flavonoids: intake, health effects and bioavailability.
Hollman, PC and Katan, MB.
Food Chem Toxicol. 1999; 37(9-10): 937-42.

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described. They may have beneficial health effects because of their antioxidant properties and their inhibitory role in various stages of tumour development in animal studies. An estimation of the total flavonoid intake is difficult, because only limited data on food contents are available. It is estimated that humans ingest a few hundreds of milligram per day. The average intake of the subclasses of flavonols and flavones in The Netherlands was 23 mg/day. The intake of flavonols and flavones was inversely associated with subsequent coronary heart disease in most but not all prospective epidemiological studies. A protective effect of flavonols on cancer was found in only one prospective study. Flavonoids present in foods were considered non-absorbable because they are bound to sugars as beta-glycosides. However, we found that human absorption of the quercetin glycosides from onions (52%) is far better than that of the pure aglycone (24%). Flavonol glycosides might contribute to the antioxidant defences of blood. Dietary flavonols and flavones probably do not explain the cancer-protective effect of vegetables and fruits; a protective effect against cardiovascular disease is not conclusive.

34. Bioactive compounds in nutrition and healthresearch methodologies for establishing biological function: The Antioxidant and Anti-inflammatory Effects of Flavonoids on Atherosclerosis.
Kris-Etherton, PM; Lefevre, M; Beecher, GR; Gross, MD; Keen, CL; and Etherton, TD.
Annu Rev Nutr. 2004; 24: 511-538.

Identifying bio active compounds and establishing their health effects are active areas of scientific inquiry. There are exciting prospects that select bioactive compounds will reduce the risk of many diseases, including chronic diseases such as cardiovascular disease. Recent findings have established that cardiovascular disease is a disease of inflammation, and consequently is amenable to intervention via molecules that have anti-inflammatory effects. In addition, research demonstrating adverse effects of oxidants on atherogenesis raises the possibility that antioxidants can confer cardioprotective effects. This review provides an overview of research approaches that can be used to unravel the biology and health effects of bioactive compounds. Because of the number of bioactive compounds and the diversity of likely biological effects, numerous and diverse experimental approaches must be taken to increase our understanding of the biology of bioactive compounds. Recognizing the complexity of this biology, sophisticated experimental designs and analytical methodologies must be employed to advance the field. The discovery of novel health effects of bioactive compounds will provide the scientific basis for future efforts to use biotechnology to modify/fortify foods and food components as a means to improve public health.

III. Mode of Action Abstracts

35. Osteoarthritis therapy--are there still unmet needs?
Laufer, S.
Rheumatology (Oxford). 2004; 43 Suppl 1: i9-15.

Non-steroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase (COX)-2 inhibitors are commonly used to control pain and inflammation in osteoarthritis. However, these agents have been associated with gastrointestinal, renal and cardiovascular adverse effects. Together, these complications indicate a clear unmet need in the safety of current treatment options for the management of osteoarthritis. NSAIDs are known to have adverse gastrointestinal effects, and more recently it has been suggested that some selective COX-2 inhibitors are also associated with serious gastrointestinal complications. Selective COX-2 inhibitors have a similar capacity to NSAIDs to delay ulcer healing, and may not significantly decrease the incidences of perforation, ulceration and bleeding (the most clinically relevant gastrointestinal endpoints) compared with NSAIDs. These effects may be due to overlapping roles of COX-1 and COX-2 in physiological and pathophysiological processes. Furthermore, as COX-2 is integrally involved in renal homeostasis, selective COX-2 inhibitors are associated with negative effects on kidney function similar to those seen with NSAIDs. Electrolyte disturbances, oedema and hypertension have been correlated with the use of both drug classes. Additionally, selective COX-2 inhibitors have the potential to increase cardiovascular events, although further research is required to clearly determine such a risk. With the current unmet needs in the treatment of osteoarthritis, the opportunity exists for the development of new therapies. Novel agents include the COX-inhibiting nitric oxide donors and the lipoxygenase (LOX)/COX inhibitor licofelone. Initial results suggest that these therapies may have tolerability advantages over the NSAIDs and selective COX-2 inhibitors.

36. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs
J Martel-Pelletier, D; Lajeunesse, P Reboul; and Pelletier J-P,
Ann Rheum Dis. 2003; 62(6): 501-9

Dual 5-LOX/COX inhibitors are potential new drugs to treat inflammation. They act by blocking the formation of both prostaglandins and leucotrienes but do not affect lipoxin formation. Such combined inhibition avoids some of the disadvantages of selective COX-2 inhibitors and spares the gatrointestinal mucosa.

37. New trends in dual 5-LOX/COX inhibition.
Leval, X; Julemont, F; Delarge, J; Pirotte, B; and Dogne, JM.
Curr Med Chem. 2002; 9(9): 941-62

Dual inhibitors are drugs able to block both the COX and the 5-LOX metabolic pathways. The interest of developing such compounds is supported by a large number of pharmacological studies. Compared to COX or LOX pathways single inhibitors, dual inhibitors present at least two major advantages. First, dual inhibitors, by acting on the two major arachidonic acid metabolic pathways, possess a wide range of anti-inflammatory activity. Secondly, dual inhibitors appear to be almost exempt from gastric toxicity, which is the most troublesome side effect of COX inhibitors. The mechanism of their gastric-sparing properties is not completely understood, although it has been demonstrated that leukotrienes significantly contribute to the gastric epithelial injury. Finally, both COX and LOX derivatives (prostanoids and leukotrienes, respectively) are involved in other diseases than inflammation such as cancer proliferation where the use of dual inhibitors could be an interesting approach.

38. Role of eicosanoids in structural degradation in osteoarthritis.
Laufer, S**.**
Curr Opin Rheumatol. 2003; 15(5): 623-7.

PURPOSE OF REVIEW: Osteoarthritis is characterized mainly by degenerative changes in joint cartilage, ultimately resulting in loss of cartilage, and alterations in the subchondral bone. Osteoarthritis osteoblasts show a number of metabolic alterations that may interfere with normal cell metabolism and signaling, possibly leading to altered extracellular matrix composition. This review examines the role of eicosanoids in this structural degradation. RECENT FINDINGS: Prostaglandins exert diverse modulatory roles in osteoarthritis, with prostaglandin E2 known to play an important role in inflammation. Prostaglandins and leukotriene B4 have been shown to regulate proinflammatory cytokine and interstitial collagenase synthesis in human osteoarthritis synovial membrane explants. Human osteoarthritis osteoblasts produce variable levels of prostaglandin E2 and leukotriene B4 compared with normal osteoblasts. Prostaglandin E2 levels can distinguish two types of patients with osteoarthritis: osteoblasts from one group produce low levels of prostaglandin E2 and interleukin-6, and the other shows an increase in production. In contrast, osteoarthritis osteoblasts that produce high levels of prostaglandin E2 produce low levels of leukotriene B4 and vice versa. This observation could be explained by the selective metabolism of arachidonic acid via the 5-lipoxygenase or cyclooxygenase pathways in osteoarthritis osteoblasts. SUMMARY: Prostaglandins play a significant role not only in joint physiology, but also in the pathogenesis of joint disorders. In addition, it has been identified that osteoarthritis subchondral osteoblasts can synthesize leukotriene B4, indicating a role of leukotrienes in bone remodeling associated with osteoarthritis. A therapeutic intervention that blocks lipoxygenase/cyclooxygenase pathways, thereby inhibiting production of prostaglandins and leukotrienes, may therefore be very attractive for the treatment of osteoarthritis patients.

39. The role of reactive oxygen species in homeostasis and degradation of cartilage.
Henrotin, YE; Bruckner, P; and Pujol, JP**.**
Osteoarthritis Cartilage. 200; 11(10): 747-55.

OBJECTIVES: The metabolism of cells in articular joint tissues in normal and pathological conditions is subject to a complex environmental control. In addition to soluble mediators such as cytokines and growth factors, as well as mechanical stimuli, reactive oxygen species (ROS) emerge as major factors in this regulation. ROS production has been found to increase in joint diseases, such as osteoarthritis and rheumatoid arthritis, but their role in joint diseases initiation and progression remains questionable. METHOD: This review is focused on the role of ROS, mainly nitric oxide, peroxynitrite and superoxide anion radicals, in the signaling mechanisms implied in the main cellular functions, including synthesis and degradation of matrix components. The direct effects of ROS on cartilage matrix components as well as their inflammatory and immunomodulatory effects are also considered. RESULTS: Some intracellular signaling pathways are redox sensitive and ROS are involved in the regulation of the production of some biochemical factors involved in cartilage degradation and joint inflammation. Further, ROS may cause damage to all matrix components, either by a direct attack or indirectly by reducing matrix components synthesis, by inducing apoptosis or by activating latent metalloproteinases.

Finally, we have highlighted the uncoupling effect of ROS on tissue remodeling and synovium inflammation, suggesting that antioxidant therapy could be helpful to treat structural changes but not to relieve symptoms. CONCLUSIONS: This review of the literature supports the concept that ROS are not only deleterious agents involved in cartilage degradation, but that they also act as integral factors of intracellular signaling mechanisms. Further investigation is required to support the concept of antioxidant therapy in the management of joint

40, Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs.
Charlier, C; and Michaux, C.
Eur J Med Chem. 2003; 38(7-8): 645-59

Dual COX/5-LOX (cyclooxygenase/5-lipoxygenase) inhibitors constitute a valuable alternative to classical non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors for the treatment of inflammatory diseases. Indeed, these latter present diverse side effects, which are reduced or absent in dual-acting agents. In this review, COX and 5-LOX pathways are first described in order to highlight the therapeutic interest of designing such compounds. Various structural families of dual inhibitors are illustrated.

41. Study of the role of leukotriene B4 in abnormal function of human subchondral osteoarthritis osteoblasts: effects of cyclooxygenase and/or 5-lipoxygenase inhibition.
Paredes, Y; Massicotte, F; Pelletier, JP; Martel-Pelletier, J; Laufer, S; and Lajeunesse, D.
Arthritis Rheum. 2002; 46(7): 1804-12

OBJECTIVE: To compare the effect of licofelone, NS-398 (an inhibitor of cyclooxygenase 2 [COX-2]), and BayX-1005 (an inhibitor of 5-lipoxygenase activating protein) on the production of leukotriene B(4) (LTB(4)) and prostaglandin E(2) (PGE(2)), and on cell biomarkers by human osteoarthritis (OA) subchondral osteoblasts. METHODS: Primary in vitro osteoblasts were prepared from subchondral bone specimens obtained from OA patients and autopsy subjects. LTB(4) and PGE(2) levels were measured by enzyme-linked immunosorbent assay in conditioned media of osteoblasts incubated in the presence or absence of licofelone, NS-398, or BayX-1005. The effect of these drugs or of the addition of LTB(4) on alkaline phosphatase (AP) activity and osteocalcin release by OA and normal osteoblasts was determined. The presence of LTB(4) receptors in normal and OA osteoblasts was evaluated by Western blot analysis. RESULTS: OA osteoblasts produced variable levels of PGE(2) and LTB(4) compared with normal osteoblasts. Licofelone, at the maximal dose used, inhibited production of PGE(2) and LTB(4) by OA osteoblasts by a mean +/- SEM of 61.2 +/- 6.4% and 67.0 +/- 7.6%, respectively. NS-398 reduced PGE(2) production by 75.8 +/- 5.3%. BayX-1005 inhibited LTB(4) production in OA osteoblasts by 38.7 +/- 14.5% and marginally affected PGE(2) levels (reduction of 14.8 +/- 5.3%). Licofelone dose-dependently stimulated 1,25-dihydroxyvitamin D-induced AP activity while inhibiting osteocalcin release. BayX-1005 partly reproduced these effects, but NS-398 failed to affect them. LTB(4) dose-dependently inhibited AP activity in OA osteoblasts, while its effect on osteocalcin depended on endogenous LTB(4) levels in these cells. In normal osteoblasts, LTB(4) dose-dependently stimulated osteocalcin, whereas it failed to influence AP. LTB(4) receptors BLT1 and BLT2 were present in normal and OA osteoblasts. CONCLUSION: Licofelone inhibits the production of PGE(2) and LTB(4). Selective effects of licofelone on AP and osteocalcin occur via its role on LTB(4) production. Because LTB(4) can modify cell biomarkers in OA and normal osteoblasts, our results suggest licofelone could modify abnormal bone remodeling in OA.

42. Enhanced gastric mucosal leukotriene B4 synthesis in patients taking non-steroidal anti-inflammatory drugs.
Hudson, N; Balsitis, M; Everit,t S; and Hawkey, CJ.
Gut. 1993; 34(6): 742-7

The effects of longstanding non-steroidal anti-inflammatory drug (NSAID) treatment on gastric mucosal synthesis of leukotriene B4 (LTB4), leukotriene C4 (LTC4), and prostaglandin E2 (PGE2) was studied. Gastric antral biopsies in 65 patients with arthritis taking NSAIDs and 23 control patients were taken and eicosanoid concentrations, stimulated by vortex mixing or calcium ionophore, were measured by radioimmunoassay. Median gastric mucosal synthesis of LTB4 was increased in patients taking NSAIDs compared with non-users: (0.9(0.2-2.5) pg/mg v 0 (0-0.6) pg/mg ($p < 0.001$)). These differences persisted when subgroups of patients

were analysed according to Helicobacter pylori colonisation or degree of mucosal injury. Synthesis of LTB4 was strongly associated with the presence of type C (chemical) gastritis. Increased synthesis of LTC4 was associated with Helicobacter pylori colonisation but not NSAID use. Synthesis of PGE2 was decreased in patients taking NSAIDs compared with control patients ($p < 0.001$). Enhanced gastric mucosal synthesis of LTB4 in patients taking NSAIDs may represent a primary effect of these drugs and could be implicated in the pathogenesis of gastritis and ulceration associated with NSAIDs.

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

Articular cartilage is one of very few body tissues uniquely characterized as having substantial stores of lipid deposits. Lipid droplets are naturally accumulated by chondrocytes and individual fatty acids have been shown to have protective as well as deleterious effects on cartilage degradation in animal models of degenerative joint disease. As a means to better assess the role of lipids in human joint pathology, a comparative analysis of fatty acids was undertaken in small segments of osteoarthritic articular cartilage. The data were assessed in terms of chondrocyte synthetic activity and histological determination of disease severity. The distribution profile of individual fatty acids in normal and osteoarthritic specimens remained constant, with palmitic, oleic, and linoleic acids representing 85% of the total fatty acids. In contrast, levels of total fatty acids were markedly increased in association with increasing degree of lesion severity. Compared with tissue from normal-aged joints, grade 0 to 1 mild lesions had elevated levels of total fatty acids, essential fatty acids, and chondrocyte synthetic activity of 80%, 312%, and 393%, respectively. More severe tissue involvement (grade 6 to 9), was associated with even greater increases of 440%, 1,100%, and 1,150%, respectively. No change was noted in cholesterol content in any tissue. The accumulation of arachidonic acid was greater than the proportional increase in total fatty acid content and was primarily distributed into the neutral lipid fraction, where it constituted almost 62% of the fatty acid level in tissues of moderate lesion severity. There was an association of lipid accumulation in general and arachidonic acid in particular with histological severity.(ABSTRACT TRUNCATED AT 250 WORDS)

IV. Other Abstracts of Interest

44. Metabolic correlates of obesity and radiographic features of knee osteoarthritis: data from the Baltimore Longitudinal Study of Aging.
Martin, K; Lethbridge-Cejku, M; Muller, DC; Elahi, D; Andres, R; Tobin, JD; and Hochberg, MC.
J Rheumatol. 1997; 24(4): 702-7.

OBJECTIVE: To examine the relationship between metabolic correlates of obesity and radiographic knee osteoarthritis (OA).

METHODS: We included 464 Caucasian men and 275 Caucasian women aged 40 years and above who were participants in the Baltimore Longitudinal Study of Aging. Subjects had bilateral anteroposterior standing knee radiographs read for features of OA using Kellgren-Lawrence scales. Resting blood pressure, fasting lipids, 2 h oral glucose tolerance test, and anthropometric measurements were obtained at the same visit as the knee radiograph. Metabolic correlates of obesity were compared between subjects with Kellgren-Lawrence grade > or = 2 (definite knee OA) and grade 0 (normalradiograph) by sex.

RESULTS: Both men and women with knee OA had higher unadjusted systolic blood pressure than those with normal knee radiographs; unadjusted measures of glucose metabolism and lipids did not vary by presence of knee OA in men or women. After adjustment for age and obesity, systolic blood pressure did not vary by presence of knee OA in men. While women with knee OA did have higher adjusted mean systolic blood pressure than women with normal radiographs (127 +/- 2.4 vs 120 +/- 2.2 mm Hg; $p = 0.04$), both values were within normal range. Unexpectedly, men with knee OA had lower adjusted mean 2 h glucose levels compared to men without OA (7.5 +/- 0.2 vs 8.4 +/- 0.2 mmol/l; $p = 0.01$). Other adjusted variables did not differ by presence of knee OA.

CONCLUSION: These data do not support the hypothesis that metabolic correlates of obesity are independently associated with radiographic knee OA after adjustment for age and obesity.

45. Prevalence of arthritis: analysis of data from the US Behavioral Risk Factor Surveillance System, 1996-99.
Mili, F; Helmick, CG; and Zack, MM.
J Rheumatol. 2002; 29(9): 1981-8.

OBJECTIVE: Arthritis and other rheumatic conditions are a large and growing public health problem and constitute the most frequent cause of disability in the United States. Because many people with arthritis do not see a doctor for it, this study uses community surveys to estimate the prevalence of arthritis among adults and to identify subgroups with high prevalence rates of arthritis.

METHODS: We used data from a cross sectional random digit telephone survey (the Behavioral Risk Factor Surveillance System) of noninstitutionalized adults aged 18 years or older conducted from 1996 through 1999. Estimates of self-reported arthritis, defined as chronic joint symptoms or doctor diagnosed arthritis, were derived from data in 15 states and Puerto Rico, all of which used an optional arthritis survey module for one or more years from 1996 through 1999. RESULTS: After adjusting for age, we found that arthritis was more common among several groups not recognized consistently in previous studies to have high prevalence rates of arthritis: separated and divorced people, those out of work or unable to work, and current and former smokers. It was also more common among several previously recognized groups with high prevalence rates of arthritis: older people, women, people with low education, people with low household incomes, physically inactive people, and overweight and obese people. CONCLUSION: Because appropriate management can minimize the influence of arthritis, health care providers should ask patients in high risk groups about arthritis symptoms. In addition, clinical and public health interventions may be targeted toward those subgroups with high prevalence rates of arthritis to reduce the disability from arthritis and improve their health related quality of life.

46. Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: the Johnston County Osteoarthritis Project.
Jordan, JM; Luta, G; Stabler, T; Renner, JB; Dragomir, AD; Vilim, V; Hochberg, MC; Helmick, CG; and Kraus, VB.
Arthritis Rheum. 2003; 48(3): 675-81.

OBJECTIVE: Previous descriptions of potential biomarkers of osteoarthritis (OA) have been limited to Caucasians. In the present study, we examined associations between serum levels of cartilage oligomeric matrix protein (COMP) and ethnicity (African American or Caucasian) and sex in the Johnston County Osteoarthritis Project, a population-based study of OA in rural North Carolina. METHODS: All African Americans and a randomly selected sample of Caucasians who had available sera and either no radiographic evidence of knee or hip OA according to the Kellgren/Lawrence (K/L) system (K/L grade 0) or radiographic evidence of knee OA (K/L grade 2 or higher) were included. Serum COMP levels were quantified by sandwich enzyme-linked immunosorbent assay, using monoclonal antibodies 16-F12 and 17-C10. Linear regression models were used to assess relationships between serum levels of natural log-transformed COMP (ln COMP) and ethnicity and sex, controlling for age, height, body mass index (BMI), radiographic OA, and the presence of other symptomatic joints. Radiographic OA was defined in separate models as the presence, severity, and laterality of radiographic knee OA, the co-occurrence of radiographic knee and hip OA, and the number of knees and hips with radiographic OA. RESULTS: The 769 subjects in the study sample had a mean +/- SD age of 62 +/- 10.3 years. Levels of ln COMP were associated with +age, BMI, and all definitions of radiographic OA (P = 0.0001), and varied by ethnicity and sex. In adjusted models, ln COMP was higher in African American women than in Caucasian women (P = 0.003) and higher in Caucasian men than Caucasian women (P = 0.0001). There were no statistically significant differences in serum ln COMP levels between African American men and women. CONCLUSION: Serum COMP levels vary by ethnicity and sex. These factors should be considered in the derivation of standards using this, and possibly other, potential biomarkers of OA.

47. Dietary modification of inflammation with lipids.
Calder, PC.
Proc Nutr Soc. 2002; 61(3): 345-58.

The n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in high proportions in oily fish and fish oils. The n-3 PUFA are structurally and functionally distinct from the n-6 PUFA. Typically, human inflammatory cells contain high proportions of the n-6 PUFA arachidonic acid and low proportions of n-3 PUFA. The significance of this difference is that arachidonic acid is the precursor of 2-series prostaglandins and 4-series leukotrienes, which are highly-active mediators of inflammation. Feeding fish oil results in partial replacement of arachidonic acid in inflammatory cell membranes by EPA. This change leads to decreased production of arachidonic acid-derived mediators. This response alone is a potentially beneficial anti-inflammatory effect of n-3 PUFA. However, n-3 PUFA have a number of other effects which might occur downstream of altered eicosanoid production or might be independent of this activity. For example, animal and human studies have shown that dietary fish oil results in suppressed production of pro-inflammatory cytokines and can decrease adhesion molecule expression. These effects occur at the level of altered gene expression. This action might come about through antagonism of the effects of arachidonic acid-derived mediators or through more direct actions on the intracellular signalling pathways which lead to activation of transcription factors such as nuclear factor kappa B (NFB). Recent studies have shown that n-3 PUFA can down regulate the activity of the nuclear transcription factor NFB. Fish oil feeding has been shown to ameliorate the symptoms in some animal models of chronic inflammatory disease and to protect against the effects of endotoxin and similar inflammatory challenges. Clinical studies have reported that oral fish oil supplementation has beneficial effects in rheumatoid arthritis and among some patients with asthma, supporting the idea that the n-3 PUFA in fish oil are anti-inflammatory. There are indications that inclusion of n-3 PUFA in enteral and parenteral formulas might be beneficial to patients in intensive care or post-surgery.

48. Identification of disease- and nutrient-related metabolic fingerprints in osteoarthritic Guinea pigs.
Lamers, RJ; DeGroot, J; Spies-Faber, EJ; Jellema, RH; Kraus, VB; Verzijl, N; TeKoppele, JM; Spijksma, GK; Vogels, JT; van der Greef, J; and van Nesselrooij, JH.
J Nutr. 2003; 133(6): 1776-80.

Osteoarthritis (OA), one of the most common diseases among the elderly, is characterized by the progressive destruction of joint tissues. Its etiology is largely unclear and no effective disease-modifying treatment is currently available. Metabolic fingerprinting provides a novel tool for the identification of biomarkers. A metabolic fingerprint consists of a typical combination of metabolites in a biological fluid and is identified by a combination of (1)H NMR spectroscopy and multivariate data analysis (MVDA). The current feasibility study was aimed at identifying a metabolic fingerprint for OA and applying this in a nutritional intervention study. Urine samples were collected from osteoarthritic male Hartley guinea pigs (n = 44) at 10 and 12 mo of age, treated from 4 mo onward with variable vitamin C doses (2.5-3, 30 and 150 mg/d) and from healthy male Strain 13 guinea pigs (n = 8) at 12 mo of age, treated with 30 mg vitamin C/d. NMR measurements were performed on all urine samples. Subsequently, MVDA was carried out on the data obtained using NMR. An NMR fingerprint was identified that reflected the osteoarthritic changes in guinea pigs. The metabolites that comprised the fingerprint indicate that energy and purine metabolism are of major importance in OA. Metabolic fingerprinting also allowed detection of differences in OA-specific metabolites induced by different dietary vitamin C intakes. This study demonstrates the feasibility of metabolic fingerprinting to identify disease-specific profiles of urinary metabolites. NMR fingerprinting is a promising means of identifying new disease markers and of gaining fresh insights into the pathophysiology of disease.

49. Dietary constituents as novel therapies for pain.
Tall, JM and Raja, SN.
Clin J Pain. 2004; 20(1): 19-26

The use of complementary and alternative medicine has dramatically increased in the United States. The effects of select dietary constituents in animal models and clinical pain states are reviewed. Specifically, the antinociceptive and analgesic properties of soybeans, sucrose, and tart cherries are discussed. The potential actions of dietary constituents as antiinflammatory and antioxidant agents are presented.