Botanical Medicine: From Bench to Bedside
Edited by R. Cooper and F. Kronenberg

Chapter 6

Curcuminoids from *Curcuma longa* in Disease Prevention and Treatment

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CHRONIC INFLAMMATION

Inflammation as a lingering rather than an acute process is increasingly important for researchers and clinicians from diversified fields of medicine. Chronic inflammation is considered an underlying pathology of many diseases that remain poorly understood or treated. Cardiovascular disease, a leading cause of mortality in the world, is no longer considered as only a disorder of lipid accumulation but also as a disease process characterized by low-grade inflammation of the endothelial cells and an inappropriate healing response of the vascular lining (1). Cancer is another chronic degenerative disease that is initiated and promoted by lingering inflammation, often triggered by environmental or nutritional factors (2). Similarly, the neurodegenerative conditions, for example, Alzheimer's disease, are hypothesized as being caused by dysfunction of the immune system reacting to chronic inflammation of the central nervous system (3).

Several epidemiological and laboratory studies have demonstrated that patients with clinically different diseases may have a similar pattern of elevated serum levels of inflammatory biomarkers including nuclear factor kappa beta (NF-κB), interleukin-6 (IL-6), cyclooxygenase enzymes (COX-1 and COX-2), tumor necrosis factor-alpha (TNF-alpha), cell adhesion molecules, for example, intercellular adhesion molecule-1 and P-selectin, and acute-phase proteins including C-reactive protein (CRP), fibrinogen, and amyloid (1, 2, 3). Dysregulation of tumor necrosis factor, or TNF, has been implicated in a wide variety of inflammatory diseases including rheumatoid arthritis, Crohn's disease, multiple sclerosis, psoriasis, scleroderma, atopic dermatitis, systemic lupus erythematosus, type 2 diabetes, atherosclerosis, myocardial infarction, osteoporosis, and autoimmune deficiency disease. TNF upregulates nuclear factor kappa beta (NF-κB is critically important as a transcription factor for the expression of TNF), and it also mediates its biological effects through activation of caspases,

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Dedicated to the pioneering work of Dr. Muhammed Majeed and Sami Labs and the Sabinsa team in introducing Ayurvedic standardized botanicals to the West.

activator protein 1 (AP-1, a transcription factor needed for expression of TNF), c-jun N-terminal kinase (JNK, which belongs to mitogen-activated protein kinases [MAPK]), p38 mitogen-activated protein kinases (p38 MAPK), and p44/p42 MAPK. These changes in levels and activity of biomarkers may signal a chronic inflammatory process in a high-risk group of patients predisposed to or suffering from a degenerative disease and cancer.

Although synthetic drugs effectively reduce inflammation and pain in both acute and chronic inflammatory conditions, they work in a very selective way that may be counterproductive to the purpose of treatment. Recent research reveals that selective COX-2 inhibitor drugs may induce metabolic imbalances that can result in the overproduction of toxic cytokines, TNF-alpha, and certain interleukins that are involved in the inflammatory process (4).

In the emerging trend to search for natural therapies, turmeric root, ginger root, rosemary leaves, green tea leaves, and their respective active phytochemical constituents are reported to be effective COX-2 inhibitors that also may inhibit the formation of inflammatory leukotrienes and toxic cytokines. These botanicals, unlike synthetic COX-2 enzyme inhibitors, do not irritate the gastrointestinal lining and generally have a safe record of traditional use spanning centuries. Furthermore, no adverse effects have been reported with these botanical preparations in clinical studies performed to validate their therapeutic properties. In fact,

Table 6.1. Comparison of Cancer Incidence in U.S. (Curcumin Non-Users) and India (Curcumin Users)

	U.S.		India	
Cancer	*Cases*	*Deaths*	*Cases*	*Deaths*
Breast	660	160	79	41
Prostate	690	130	20	9
Colon/rectum	530	220	30	18
Lung	660	580	38	37
Head and neck SCC	140	44	153	103
Liver	41	44	12	13
Pancreas	108	103	8	8
Stomach	81	50	33	30
Melanoma	145	27	1.8	1
Testis	21	1	3	1
Bladder	202	43	15	11
Kidney	115	44	6	4
Brain, nervous system	65	47	19	14
Thyroid	55	5	12	3
Endometrial cancers	163	41	132	72
Ovary	76	50	20	12
Multiple myeloma	50	40	6	5
Leukemia	100	70	19	17
Non-Hodgkin's lymphoma	180	90	17	15
Hodgkin's disease	20	5	7	4

Showing cases per 1 million persons calculated on the basis of current consensus: endometrial cancers include Cervix uteri and Corpus uteri.

Source: GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARC Cancer Base No. 5. Lyon, IARC Press, 2001.

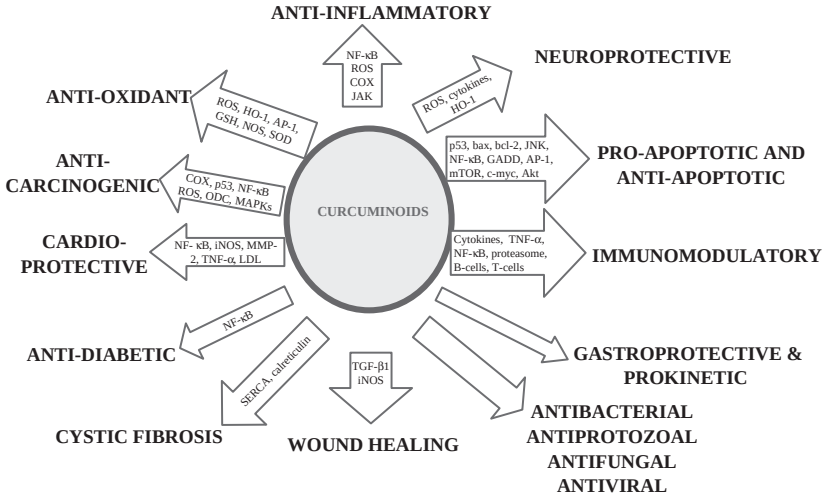


Figure 6.1. Mode of Action of Curcuminoids

Acknowledgment: Gescher Andy, University of Leicester, UK

many of the compounds found in the extracts are on the U.S. Food and Drug Administration (FDA) GRAS (generally recognized as safe) list of compounds that are safe to use in daily nutrition. In this regard, based on the current body of scientific evidence, turmeric's (curry) curcuminoids are considered the most promising food-derived compounds to fight inflammation and related diseases (5, 6) (Table 6.1).

There is a significant amount of research conducted on the health benefits of curcuminoids summarized in Figure 6.1. In this chapter the potential for curcuminoids in inflammatory processes will be discussed. In view of the efficacy and apparently low toxicity of curcuminoids, securing a predictable delivery of curcuminoids to the target tissue(s) will be essential to fulfill their promise for medicinal food or novel drug applications in prevention and treatment of inflammatory and neoplastic diseases.

STANDARDIZATION OF TURMERIC'S CURCUMINOIDS

Curcumin (chemically diferuloylmethane) and its derivatives demethoxycurcumin and bisdemethoxycurcumin, collectively known as curcuminoids, are responsible for the yellow pigment derived from the roots of the perennial herb turmeric (*Curcuma longa* L. Family *Zingiberaceae*) (Figure 6.2). The same ground, dried roots of turmeric, which have been used for centuries as a spice (curry), a food preservative, and a coloring agent, have been found to be a rich source of phenolic compounds (curcuminoids) with versatile biological mechanisms (6) and (6a). In dietary supplement practice and in a growing body of scientific research, an extract of turmeric roots is now being utilized that is standardized for a high purity of curcuminoids, for example, 95% curcuminoids, devoid of environmental contaminants (7–22). The standardization is achieved on the basis of the content of a group of bioactives, that is, curcuminoids, by a high-pressure liquid chromatography (HPLC) method, resulting in the following combination:

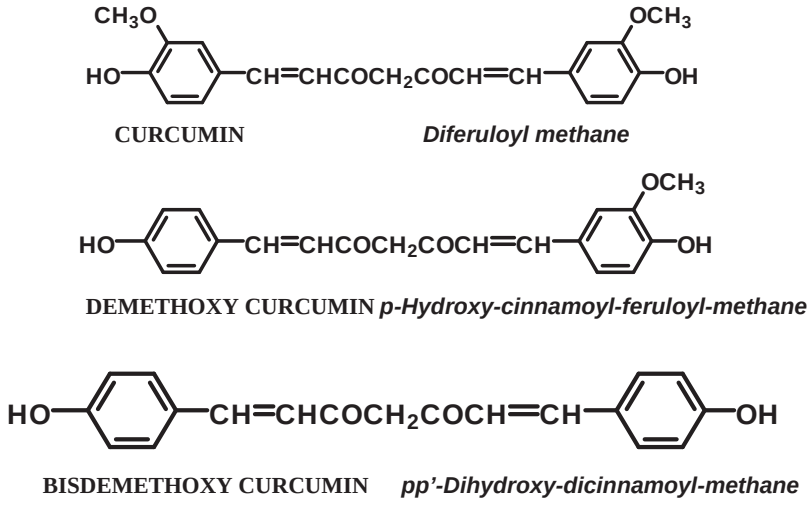


Figure 6.2. Chemical Structures of Curcuminoids

(total curcuminoids not less than 95.0% calculated on dried basis)
(bisdemethoxy curcumin not less than 2.5% and not more than 6.5%)
(demethoxycurcumin not less than 15.0% and not more than 25.0%)
(curcumin not less than 70.0% and more than 80.0%)

but also based on limits for various quality parameters such as the ash content (no more than 1.0%), melting range (172–178°C), bulk density (0.5 g/ml), sieve values (standard size of the particles of the extract), solubility in water (insoluble in water) and organic solvents (slightly soluble in alcohol, soluble in acetone and glacial acetic acid), microbial (total microbial count < 100 cfu/g and absence of pathogenic bacteria) and mold and yeast (< 10 cfu/g) loads, residual solvents from the extraction process, and pesticide (e.g., in Japan there is a list of 183 pesticides for which curcuminoids have to test negative) and aflatoxin levels (have to be < 20 ppb).

The limits for heavy metals (total heavy metals under 10 ppm, arsenic < 1 ppm, lead < 0.5 ppm) are an important quality control and standardization parameter. There are two possible sources of heavy metals in the turmeric extract. The first one is the plant's ability to absorb from the soil, that is, "naturally occurring." The second is contamination from the process (equipment, process water, and the solvents and chemicals used in the manufacture).

CURCUMINOIDS IN TREATMENT OF CANCER

Potential Mechanisms of Action

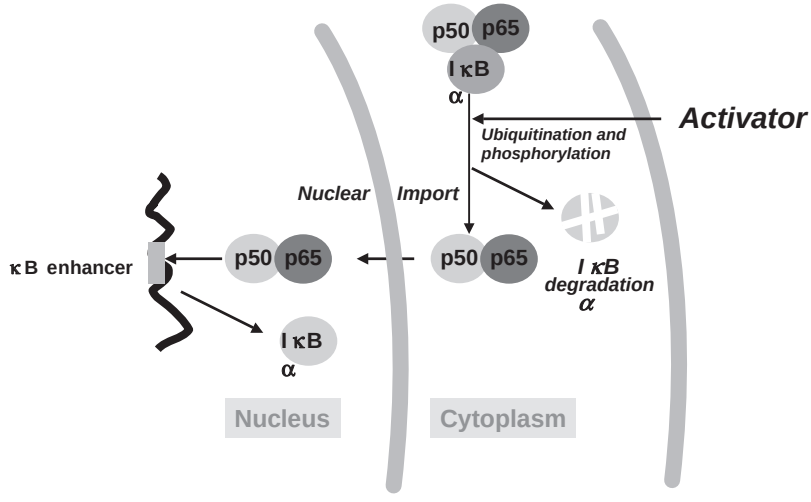
In the last three years alone, several pioneering IND (Investigational New Drug) studies have been granted by the FDA and other studies funded by the National Institutes of Health to investigate curcumin and its derivatives in the treatment of patients with cancer (16, 17, 18). Some of the leading clinical research centers in the United States, including MD

Anderson Hospital in Houston, Texas, are involved in preclinical and clinical research of the anticancer mechanism and application of curcuminoids in conditions including multiple myeloma and colon, pancreatic, breast, prostate, head and neck, and respiratory tract cancers. These cancer conditions are either currently being studied in clinical experiments or considered next in line for systematic evaluation with curcuminoid therapy.

Curcuminoids act by inhibiting several processes that contribute to the survival, proliferation, invasion, and metastasis of tumor cells (23). Curcuminoids act by interfering with signaling mechanisms (critical for tumor growth), regulation of apoptosis, and tumor angiogenesis. Current research is designed to determine which of these fundamental processes in cancer development account for the clinical effects of curcumin and its derivatives.

Curcuminoids have significant immunomodulating and anti-inflammatory effects, in part caused by the inhibition of cyclooxygenase type 2 enzyme (COX-2) and its subsequent arachidonic acid metabolism (24, 25). Curcuminoids, like several other immunomodulators, inhibit the activation of the nuclear factor kappa-B (NF-κB) family of transcription factors that are known to be activated in a wide variety of solid tumors, lymphomas, and leukemias (26, 27, 28) (Graph 6.1, Table 6.2). The activation of NF-κB may shield tumor cells from apoptosis, or programmed cell death, and promote tumor growth factors and those factors that facilitate invasion and metastasis of tumors. Curcuminoids block the NF-κB–mediated gene expression responsible for the chain of events leading to tumor development, progression, and expansion. A probable mechanism of curcuminoids appears to be blocking the degradation of the inhibitors of NF-κB (Graph 6.1; 26). In vitro, curcuminoids induce apoptosis and thus inhibit tumor growth in a broad range of tumor cells, including cell lines from colon, breast, prostate, squamous cell, renal cell, and hepatocellular carcinomas; B- and T-cell lymphomas; leukemias; melanoma; and sarcoma cells (29).

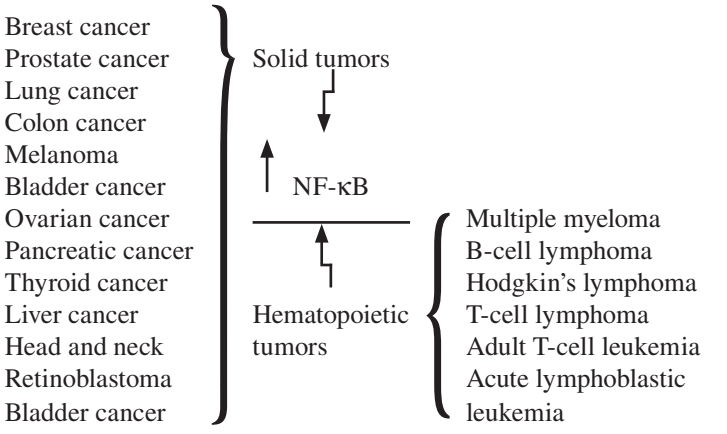
Curcuminoids also affect a signaling mechanism that involves expression and activation of certain growth factor receptors that promote tumor growth. For example, HER-2/neu is



Graph 6.1. What Is NF-κB?

Source: *Leukemia*. 2002 Jun;16(6):1053–1068

Table 6.2. Tumors Linked to NF-κB Expression



Source: *Curr. Opin. Oncol.* 2003; 15(6): 405–411.

a member of the Epidermal Growth Factor Receptor family, which is overexpressed in approximately 30% of breast cancer patients. HER-2/neu–positive breast cancer cells, when exposed to curcumin, were found to have decreased expression of the HER-2 receptor (29, 30, 31).

This ability makes curcumin a promising agent for combination with paclitaxel (Taxol) (29). The drug Taxol is derived from the Pacific yew tree and is produced semisynthetically using a combination of fermentation and synthesis. It is used as first-line chemotherapy in breast cancer. It induces the apoptosis of breast tumor cell lines, but overexpression of HER-2/neu may block these apoptotic effects and induce resistance to Taxol. Further, HER-2/neu and Taxol can activate antiapoptotic pathways through activation of NF-κB. Thus, agents such as curcuminoids that can down-regulate activation of NF-κB and decrease HER-2/neu overexpression and other markers of tumorigenesis may augment the therapeutic effects of Taxol against breast cancer (Figure 6.3).

Interestingly, curcuminoids may have a comparable mechanism of action to the drug therapy involving Herceptin for breast cancer patients with HER-2 receptor–positive cancer cells. Herceptin is an antibody against HER-2 receptors, binding, blocking, and inactivating those receptors. In vitro, the growth of breast cancer cells with multidrug resistance (MDR) characteristics is inhibited by these turmeric phenolics; the stimulation of estrogen receptor (ER)–positive cell lines by estrogenic pesticides is also inhibited by curcuminoids (31, 32). Curcuminoids have also been found to inhibit epidermal growth factor receptor expression or activation in skin cancer cell lines as well as in androgen-sensitive and androgen-insensitive prostate cancer cell lines (33).

An important anticancer mechanism of curcuminoids is restriction of vital blood supply to the rapidly growing tumor (34, 35). In vitro, these compounds inhibit the blood vessel endothelial and smooth muscle cell growth and proliferation, which is the basis for inhibition of angiogenesis (new blood vessel formation). Curcuminoids also inhibit new vessel formation induced by growth factors, such as fibroblast growth factor-2 (FGF-2) (34). Furthermore, curcuminoids inhibit the production of vascular endothelial growth factor (VEGF) in human melanoma cells (36). The antiangiogenic effect of turmeric compounds

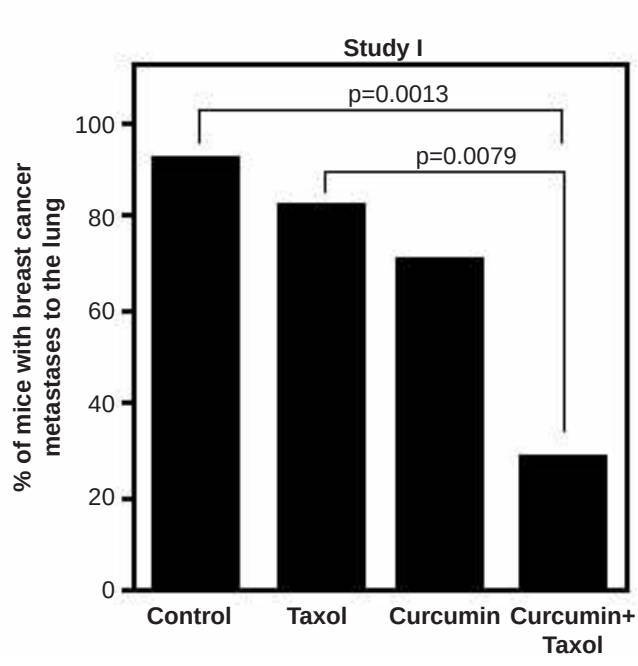


Figure 6.3. Effect of Curcumin, Taxol, or Their Combination on Breast Cancer Metastasis to the Lung

Source: *Clin Cancer Res.* 2005 Oct 15;11(20):7490–7498.

can be explained by the aforementioned selective COX-2 inhibition with curcuminoids. COX-2 enzyme activity may contribute to tumor growth (inhibition of apoptosis) along with increased production of the new vessel growth factors (VEGF, FGF) and the formation of new blood vessels. In in vitro experiments, COX inhibitors inhibited the growth of human colon, prostate, gastric, lung, head and neck, pancreatic, liver, and breast cancer cell lines (37, 38).

CLINICAL TRIALS IN CANCER

Human clinical trials with curcuminoids have been undertaken in several cancers in view of evidence of safety and efficacy in preclinical studies of these compounds in prevention and treatment of several forms of cancer. Several examples of clinical trials against various cancers are discussed. The data include design, use of biomarker measurements, adverse events, and a discussion of dosing and clinical outcome.

Clinical Trials in Patients with Colon Cancer

Gastrointestinal cancer has been of particular interest because of its epidemiological significance. Curcuminoids were evaluated in the Department of Oncology, University of Leicester, United Kingdom, in a dose-escalation study in patients with advanced colorectal cancer refractory to standard chemotherapy (7). The primary objective of this four-month study was to evaluate markers of efficacy of the novel chemopreventive agent. Three biomarkers of the potential activity of curcuminoids were measured in the peripheral blood

leukocytes: glutathione *S*-transferase activity (GST), levels of M1G (DNA adduct [M1G] formation), and prostaglandin E2 (PGE2) production induced ex vivo.

Design of the Trial

Fifteen patients with histologically proven adenocarcinoma of the colon or rectum for which no additional conventional therapies were available were selected for the study based on the following criteria: measurable or evaluable disease; age > 18 years; World Health Organization (WHO) performance status of 0 to 2 and life expectancy > 12 weeks; absolute neutrophil count > 1.5 × 109/L; hemoglobinn > 10 g/dL; platelets > 100 × 109/L; aspartate aminotransferase and alanine aminotransferase < 2.5 times the upper limit of normal; serum bilirubin and creatinine < 1.5 times the upper limit of normal; and no previous investigational or chemotherapeutic drugs within 28 days before enrollment. Patients were asked to abstain from nonsteroidal anti-inflammatory drug use and the consumption of foods containing the spice turmeric during the study period, and their general practitioners were asked not to prescribe nonsteroidal anti-inflammatory drugs. Written informed consent was obtained from each patient before enrollment. All of the patients were Caucasians except for one patient who was Indian. The trial and formulation were approved by the local ethics committee and the United Kingdom Medicines Control Agency.

Each patient was assigned to the dose level (DL), and depending on dose level, patients consumed one, two, four, or eight capsules, providing 450 (DL1), 900 (DL2), 1800 (DL3), or 3600 (DL4) mg of curcuminoids, respectively, once daily. The capsules of a daily dose of curcuminoids were consumed together with water in the morning after at least two hours of fasting. Treatment was continued until disease progression was established or consent was withdrawn. The three indices of the potential pharmacological activity of curcumin measured in patient blood leukocytes were GST activity, levels of a deoxyguanosine adduct (M1G) formed via oxidative DNA damage, and inducible prostaglandin E2 (PGE2) levels as an indicator of COX-2 activity induced ex vivo by lipopolysaccharide (LPS). Serum levels of total cholesterol and the tumor markers carcinoembryonic antigen CA19.9 and CA125 were measured before treatment and every month of treatment. Blood samples for analysis of GST activity and M1G levels were collected one week before and on days 1, 2, 8, and 29 of treatment, immediately before dosing for M1G or immediately before and one hour after each dose for GST. Blood was also taken for assessment of plasma PGE2 concentration induced ex vivo.

Patients were evaluated for tumor response every eight weeks, using computed tomography or magnetic resonance imaging scanning, in addition to monthly chest X-rays. Measurements were made using the WHO Solid Tumor Response Criteria. Partial response was defined as at least a 50% decrease in measurable lesions from baseline and with no development of new lesions. Progressive disease was defined as at least a 25% increase, clear worsening from previous assessment of any evaluable disease, reappearance of any lesion that had disappeared, or appearance of any new lesion or site. Stable disease was defined as the scenario in which the disease status had neither responded to meet the partial response criterion nor progressed to meet the progressive disease criteria.

Tolerance and Side Effects

Curcuminoids were well tolerated at all of the dose levels. Two types of gastrointestinal adverse events were reported by patients, which were probably related to curcuminoids' consumption. One patient consuming 0.45 g curcuminoids daily and one patient consuming

3.6 g curcuminoids daily developed diarrhea (National Cancer Institute grades 1 and 2) one month and four months into treatment, respectively. In the first case, diarrhea was controlled with an antidiarrheal drug. The other patient withdrew consent from the study, and the diarrhea resolved after cessation of treatment. One patient consuming 0.9 g curcumin daily experienced nausea (National Cancer Institute toxicity grade 2), which resolved spontaneously despite continuation of treatment. Two abnormalities were detected in blood tests, both possibly related to treatment: a rise in serum alkaline phosphatase level was observed in four patients, consistent with National Cancer Institute grade 1 toxicity in two patients and grade 2 toxicity in two patients; serum lactate dehydrogenase levels rose to > 150% of pretreatment values in three patients.

Clinical Status

All of the patients enrolled exhibited radiologic evidence of progressive disease before recruitment. No partial responses to treatment were observed. Two patients exhibited stable disease by radiologic criteria after two months of treatment, and they remained on treatment for a total of four months. The first of these two patients (DL2) developed progressive disease on her second computed tomography scan. The other patient (DL3) demonstrated continued stable disease on a computed tomography scan after four months, but she withdrew consent on account of diarrhea, which she thought was treatment related. Decreases in tumor markers were not observed as a result of treatment in any of the patients. Three significant changes in quality of life scores were recorded: one patient noticed a significant improvement after one month of treatment, and two patients deteriorated after two months of treatment, both of whom were found to have radiologic progressive disease.

Biomarkers of the Trial

Oral administration of curcuminoids did not impact on basal PGE2 levels in leukocytes, nor did doses of 0.45 to 1.8 g daily alter LPS-induced PGE2. However, consumption of 3.6 g of curcumin daily affected LPS-induced PGE2 levels. When values obtained immediately predose or one hour postdose on days 1, 2, 8, and 29 were pooled for the six patients consuming this dose, PGE2 levels observed postdose were significantly lower, 46%, than those measured immediately predosing ($p = 0.028$). There was no time-dependent trend in basal or LPS-stimulated PGE2. A subset analysis revealed no difference between inducible PGE2 levels in samples from the three patients in which curcumin was detected compared with those in which curcumin was not detected. These results suggest that consumption of 3.6 g of curcumin daily is linked with inhibition of PGE2 induction in blood taken postdose compared with blood taken predose. Oral curcuminoids at any dose level showed no effect on GST and M1G formation.

Levels of Curcumin and Derivatives in Plasma, Urine, and Feces

Curcumin was detected in plasma samples taken 0.5 and 1 hour postdose from patients consuming 3.6 g of curcumin daily. Glucuronides and sulfates of curcumin and desmethoxycurcumin were found in the plasma from all patients consuming 3.6 g of curcumin daily at all of the time points studied. Curcumin and its glucuronide and sulfate metabolites were detected in plasma in the 10 nmol/L range. Analysis of urine suggested the presence of curcumin and the conjugates in all of the samples from patients consuming 3.6 g of curcumin daily. Using HPLC, such chromatographic peaks were not seen in any extracts of urine samples from patients on the lower doses. The HPLC techniques demonstrated the

presence of curcumin and curcumin glucuronide, desmethoxycurcumin and desmethoxycurcumin glucuronide, and curcumin sulfate in urine. Abundant amounts of curcumin were recovered from the feces at all of the dose levels. Trace amounts of curcumin sulfate were detected in feces from three patients consuming 3.6 g of curcumin daily.

The study presented herein provides the first report of the systemic parameters of pharmacokinetics and activity of curcuminoids that may facilitate the phase II chemoprevention or anticancer trials. The results allow the following conclusions regarding oral curcuminoids in humans: (a) administration of 0.5 to 3.6 g daily for up to four months is associated with mild diarrhea in colon cancer patients as the only reported side effect of the treatment; (b) consumption of 3.6 g of curcuminoids daily generates detectable levels of parent compound and conjugates in plasma, urine, and feces; and (c) consumption of 3.6 g of curcumin daily causes inhibition of PGE2 production in blood leukocytes measured ex vivo.

In conclusion, a daily oral dose of 3.6 g of curcuminoids is advocated for phase II evaluation in the prevention or treatment of colorectal cancer. PGE2 blood levels may indicate the biological activity; however, the possibility of induction of GST enzymes and suppression of oxidative genetic material damage (DNA adduct [M1G] formation) with curcuminoids should not be discounted.

Clinical Trial in Patients with Pancreatic Cancer

In a clinical trial conducted at MD Anderson Medical Center, Houston, Texas, curcuminoids were evaluated in treatment of patients with pancreatic cancer. Pancreatic cancer is one of the most lethal cancers, with an overall five-year survival rate of < 5%, with most patients dying of the disease within one year. The poor prognosis of pancreatic cancer is due to its tendency for late presentation; aggressive, local invasion; early metastases; and poor response to chemotherapy. The only drugs approved by the FDA for treatment of pancreatic cancer are gemcitabine and erlotinib. Both of these drugs elicit responses in only a small percentage of patients (fewer than 10%), their effect on survival is measured in weeks, and treatment is associated with multiple adverse effects and drug resistance. There is a need for novel strategies involving less toxic agents that can sensitize pancreatic cancer cells to chemotherapy.

Several in vitro and in vivo studies have shown that nuclear transcription factor-κB (NF-κB) is activated in an experimental model of pancreatic cancer. The transcription factor has been linked with cell proliferation, invasion, angiogenesis, metastasis, suppression of apoptosis, and chemoresistance in multiple tumors including pancreatic cancer. The preclinical studies have shown that curcuminoids suppress NF-κB activation and the growth of human pancreatic cancer xenografts in mice. Phase I human clinical trials of curcuminoids have shown that curcuminoids are safe at doses of up to 8 g per day (39). Subsequently, the phase II clinical trial described below was undertaken to determine whether orally administered curcuminoids have biological activity in patients with advanced pancreatic cancer (40).

Design of the Trial

This trial was a nonrandomized, open-label, phase II clinical study. Twenty-five patients (13 men, 12 women; aged 43–77) with histologically confirmed pancreatic cancer and a Karnofsky performance score greater than 60 but preserved hepatic function and renal

function were enrolled in the phase II trial. Patients took 8 g of curcuminoids in capsule form daily for up to 18 months. The patients could not receive any concomitant chemotherapy and radiotherapy. Patients who were diagnosed as at least with stable disease after eight weeks received continued therapy with curcuminoids at 8 g per day.

A physical examination, an electrocardiogram, a chest X-ray, diagnostic imaging, and disease staging were performed at baseline, repeated every four weeks and at the end of therapy for all patients enrolled in the study; diagnostic imaging was repeated every eight weeks during the course of treatment. Blood samples were used to measure the following values: cytokine concentrations (interleukin-6, -8, and -10 and interleukin-1 receptor antagonist [IL-1RA]), tumor markers (CA 19-9, CA 27.29, CA 125, and carcinoembryonic antigen) concentrations, and peripheral blood mononuclear cell expression of NF-κB, COX-2, and signal transducer and activator of transcription 3 (pSTAT3). Blood samples were collected pretherapy at 24 hours, 8 days, 4 weeks, and 8 weeks and analyzed using enzyme-linked immunosorbent assay (ELISA) and electrophoretic mobility shift assay (EMSA), assessed for pharmacokinetics of curcuminoids. The adverse events were assessed on the basis of the National Cancer Institute Expanded Common Toxicity Criteria, and tumor response was evaluated on the basis of the Response Evaluation Criteria in Solid Tumors.

Clinical Status

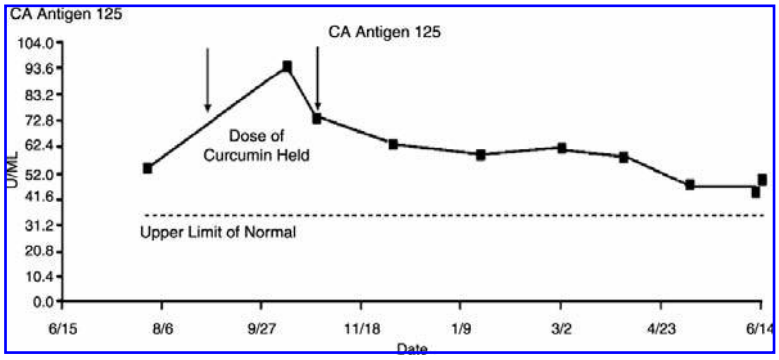
Twenty-four patients were available for the toxicity evaluation, and 21 patients were available for evaluation of the response to treatment with curcuminoids. The breakdown of non-evaluable patients was as follows: one patient developed gastric obstruction on day 1, one patient was noncompliant, and two patients were lost because of inadequate follow-up.

There were no treatment-related adverse effects. Two of the 21 patients exhibited a favorable response to curcuminoids. In addition, a third patient remained on the study for approximately eight months with stable weight and a feeling of well-being—however, with progression of the lesions. Pancreatic cancer remained stable in one of the aforementioned patients for longer than 18 months. This patient was previously treated with failed Whipple's surgery followed by gemcitabine and radiation therapy. The patient had an elevated CA 125 but not CA 19.9 level. With the treatment of curcuminoids, the CA 125 antigen levels decreased gradually over the course of 12 months (Graph 6.2).

The first patient remained in relatively good physical condition, maintained his body weight, and did not develop ascites or edema. His neoplastic lesions remained stable in size as evaluated by serial positron emission tomography or computer tomography scans. The second patient had a marked response that lasted one month and was manifested by 73% reduction in the tumor size. Curiously, once the tumor progression started again, the lesions that had regressed remained small, but other lesions grew larger.

Biomarkers of the Trial

The baseline serum cytokines were elevated in the majority of the patients. In contrast, the majority of healthy volunteers had undetectable serum levels of IL-6, IL-8, and IL-10, with all having detectable serum levels of IL-IRA. Interestingly, the two patients who had a positive response to curcuminoids had their baseline IL-6 below the median levels and the IL-1RA above the median levels; the patient who benefited most, that is, had over 18 months of gradual improvement, had the highest initial IL-1RA level among all participants of the study. Treatment resulted in variable changes in cytokine levels. Of particular interest was



Graph 6.2. Pancreatic Cancer Trial: CA125 [Cancer Antigen 125] Levels in a Patient

Serum levels of CA125 increased before dropping again when curcuminoids were administered. CA 19-9 was not elevated.

Source: *Clin Cancer Res.* 2008 Jul 15;14(14); 4491–4499.

the patient with brief and dramatic regression of tumor who now had a significant increase in serum levels of all measured cytokines, for example, the in-treatment IL-6 level that reached 35-fold of the baseline level for this patient. One way to explain the surge in cytokines is release of cytokines from the tumor associated with shrinkage of the tumor mass. Of potential importance in this patient is the observation that the tumors that originally had regressed continued to show regression in follow-up, whereas the tumors that grew previously had been dormant. This finding suggests that there was a malignant clone that responded to curcuminoids, while another resistant clone emerged. The patient who had the stable disease condition for more than 18 months experienced gradual decrease in all cytokine levels over time. As previously mentioned, this patient had the highest baseline levels of IL-1RA of the tested patients. This finding may be biologically relevant because IL-1RA opposes IL-1, which can stimulate growth of pancreatic cell lines in vitro.

The NF-κB and the activated-by-NF-κB COX-2 play critical roles in the growth and angiogenesis of pancreatic cancer. Immunocytochemistry of peripheral blood mononuclear cells (PBMC) showed constitutively active NF-κB in all patients who consented to optional tests (19 out of 25 patients), whereas activation of the transcription factor was not detected in healthy volunteers. On treatment with curcuminoids, immunocytochemistry values showed a decline (without reaching statistical significance $p=0.1$) in cellular expression of NF-κB compared with that in normal volunteers. PMBC in all 19 patients examined expressed COX-2, which declined significantly ($p<0.03$) in the course of treatment with curcuminoids. The expression of NF-κB correlated well with the expression of COX-2, and the majority of the patients showed down-regulation of NF-κB and COX-2 after treatment with curcuminoids. This is the first study to show that curcuminoids can down-regulate the expression of NF-κB and COX-2 in humans. Nevertheless, the down-regulation was not associated with clinical response in many patients, possibly because the down-regulation in PBMC does not reflect the changes in the tumor itself.

The expression of activated pSTAT3 in PBMC was also evaluated, because this compound is regulated by growth factors, for example, epidermal growth factor, and it is implicated in tumorgenesis and chemoresistance. All tested patients had activated STAT3 at

baseline, and there was a statistically significant decline ($p = 0.009$) in the percentage of pSTAT3-positive PBMCs in course of treatment with curcuminoids.

Clinical Trial in Patients with Pancreatic Cancer

Another study performed at the Department of Oncology, Rambam Medical Center, Haifa, Israel, was undertaken to evaluate feasibility and efficacy of gemcitabine in combination with curcuminoids in patients with advanced pancreatic cancer (41). Patients received 8 g of curcuminoids by mouth daily, concurrently with gemcitabine 1000 mg/m2 IV weekly times three out of four weeks. Time to tumor progression was the primary end point and the toxicity profile the main secondary end point. Seventeen patients (ten male, seven female, aged 54–78) were enrolled for the study. Six patients had locally advanced tumors and 11 patients had metastatic disease, all in the liver. Patients received a median of two cycles of gemcitabine. Five patients discontinued curcuminoids after a few days to two weeks because of intractable abdominal fullness or pain. One patient died unrelated to the treatment event. In the 11 patients, curcuminoids and gemcitabine were delivered concomitantly for a period of 1 to 12 months. The dose of curcuminoids was reduced to 4 g per day in three of the patients because of abdominal discomfort. One patient out of the 11 evaluable patients had partial response (7 months), four had a stable disease condition (2, 3, 6, and 12 months), and six had tumor progression. Time to tumor progression was 1 to 12 months (median 2) and overall survival 1 to 24 months (median 6). These preliminary results suggest that a combination of gemcitabine and curcuminoids for patients with advanced pancreatic cancer is feasible. Based on the outcome of this study, a clinically acceptable daily oral dose of curcuminoids in pancreatic cancer patients on chemotherapy should be less than 8 g per day.

Clinical Trials in Cancer-Predisposing Plasma Cell Dyscrasias

The hypothetical role of curcuminoids as chemopreventive agents in management of plasma cell dyscrasias has been studied in a clinical trial at St. George Hospital, Sydney, Australia. The trial assessed a potential therapeutic effect of curcuminoids in patients diagnosed with monoclonal gammopathy of undefined significance (MGUS) (19).

Plasma cell dyscrasias, most commonly associated with paraproteinaemia, are a diverse group of disorders that includes multiple myeloma, Waldenstrom's macroglobulinaemia, heavy chain disease, MGUS, and immunocytic amyloidosis. The incidence of plasma cell dyscrasias is age related. It occurs in 1% of the population over age 25 and 4% of those over age 70.

MGUS is the most common of the monoclonal gammopathies. While this condition occurs in association with a variety of diseases, it can also precede the onset of multiple myeloma. MGUS patients have a serum M-protein value of < 30 g/L, fewer than 10% plasma cells in the bone marrow, no or a small amount of M protein in the urine, and absence of lytic bone lesions, anemia, hypercalcemia, or renal insufficiency related to the plasma-cell proliferative process. MGUS is largely considered a benign condition; however, a number of studies show that patients with MGUS are at increased risk of developing fractures even before progression to multiple myeloma. Multiple myeloma is a progressive

neoplastic disease and is characterized by high bone turnover, significant bone loss, and pathological fractures resulting in significant morbidity and a high mortality. It is also associated with hypercalcemia, anemia, renal damage, and increased susceptibility to bacterial infections.

Current management of MGUS patients includes the regular clinical observation for changes in clinical and immunochemical status at four- to six-month intervals. Overall, the risk of progression of MGUS to myeloma or related disorder is 1% per year. Younger patients are more likely to have progression to cancer during their lifetime because they are at risk longer. It is currently not possible to predict the course in any individual patient, and clinically, symptomatic myeloma may not evolve for as long as 20 years.

Because disease progression with MGUS is uncertain, early intervention to reduce the paraprotein load and the potential negative effects on the skeleton would provide an innovative therapeutic tool. Curcuminoids were selected for the trial with MGUS patients because numerous reports suggest that curcumin and its derivative curcuminoids have chemopreventive and chemotherapeutic effects. Curcumin has been shown to inhibit the proliferation of multiple myeloma cells through the down-regulation of IL-6 as well as to induce apoptosis in multiple myeloma cells. Curcumin has also been shown to inhibit osteoclastogenesis through the suppression of the receptor activator of nuclear factor kappa B ligand (RANKL is the primary mediator of osteoclast formation, function, and survival) signaling and subsequently to reduce bone turnover.

Design of the Trial

In a single-blind, randomized control pilot study, curcuminoid formula or placebo was administered orally, 2 g (2 caplets each 1000 mg curcuminoids) twice daily to a cohort of 26 MGUS patients. Patients were asked to abstain from drugs affecting bone metabolism for at least three months prior to and during the study. No patients had evidence of metabolic bone disorder. All the patients were Caucasian, aged over 45 years. The study design was approved by the local ethics committee. Written informed consent was obtained from each patient before enrollment.

Blood and urine samples were collected at baseline (V1), one week (V2), one month (V3), and three months (V4) after initiating the therapy. Full blood count, B2 microglobulin, serum paraprotein, and immunoglobulin electrophoresis (IEPG and EPG) were determined for all patients at each visit. Serum calcium, 25 hydroxyvitamin D3, and bone-specific alkaline phosphatase (bsALP) were determined at baseline only. Urine, as a morning second-void sample, was collected at each visit for uNTx (urinary N-telopeptide of type I collagen) measurements.

Clinical Status: Paraprotein Levels

Of the 26 patients randomized into the study—16 men and 10 women with average age of 68 years—24 patients completed the study. Seven (of the 17) patients were administered curcuminoids only during the course of the study. Two patients withdrew from the study before crossover, as they developed diarrhea, which resolved after cessation of treatment. The other five patients elected to remain on curcuminoids and refused crossover.

Baseline clinical data and serum biochemistry of the patients who completed the study are summarized below. Serum paraprotein concentration ranged from 8 to 36 g/L, with a median value of 20 g/L. Nine of the 26 patients randomized into the study had IgGk (35%), nine had IgGl (35%), four had IgMk (15%), three had IgAk (11%), and one had IgAl

(4%) paraproteins. All patients had a normal serum calcium level (mean = 2.62 mmol/L). There were nine patients with vitamin D deficiency (< 50 nmol/L). Two patients had elevated baseline serum bone-specific alkaline phosphatase measurements. All patients had elevated baseline B2 microglobulin levels (mean = 2.9 ± 1 mg/L), which remained unchanged throughout the study. All patients had normal baseline uNTx levels (mean of 27 ± 16 nmol/mmol creatinine), with no patient having a baseline value greater than 100 nmol/mmol creatinine.

Of the 17 patients who were started on curcuminoids, 10 had a baseline serum paraprotein level greater than or equal to 20 g/L (mean = 20.2 g/L) and 7 less than 20 g/L. In patients with a serum paraprotein ≥ 20 g/L, 50% of these (i.e., five patients) had a 5% to 30% decrease in serum paraprotein levels in response to curcumin. The most significant decrease was seen at V2 ($p < 0.05$ for group comparison between V1 and V2) (Figure 6.4). This decrease remained stable in most patients until they were crossed over to placebo. Two patients then demonstrated a rebound in their serum paraprotein levels. Patients with a baseline serum paraprotein less than 20 g/L did not show a response to curcuminoids, but their serum paraprotein levels remained stable throughout the study period.

Nine patients were randomly assigned to receive placebo at baseline. In contrast to a decrease in serum paraprotein seen in patients initiating curcuminoid therapy, patients receiving placebo demonstrated stable or increased serum paraprotein levels at V2 (Figure 6.5). At V4 (i.e., crossover), two patients demonstrated a decrease in their serum paraprotein.

Clinical Status: uNTx Levels

While 73% of patients did not show a change in their uNTx levels while taking curcuminoids or placebo, 27% of patients showed a decrease in their uNTx levels when taking curcuminoids. This response was most marked in two patients at crossover. Although the difference between the groups (i.e., curcuminoids vs. placebo) does not reach statistical significance, the results do indicate that certain patients may show a decrease in bone resorption in response to curcuminoids.

The present study demonstrates that oral curcuminoids are able to decrease paraprotein load and bone resorption in a select group of patients with MGUS. Fifty percent of patients

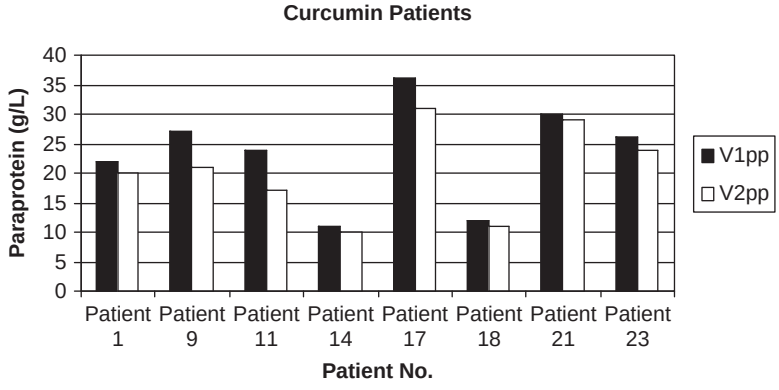


Figure 6.4. MGUS Trial: Paraprotein Levels in Patients Receiving Curcuminoids

Source: *Clin Cancer Res.* 2008. In press.

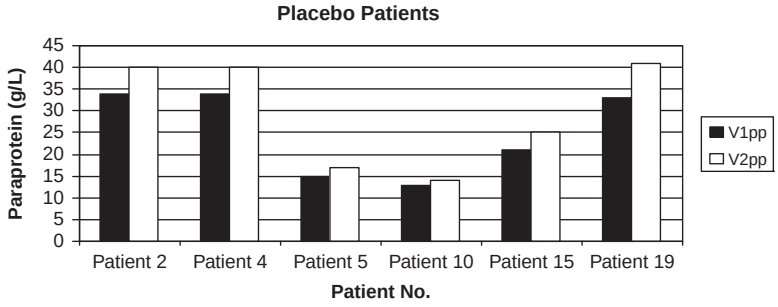


Figure 6.5. MGUS Trial: Paraprotein Levels in Patients on Placebo

Source: *Clin Cancer Res.* 2008. In press.

with a paraprotein ≥ 20 g/L responded with a 5% to 30% decrease in their paraprotein levels when taking 4 g per day curcuminoids orally. This response occurred already after seven days of curcuminoid therapy. Patients taking placebo had no such decrease in their paraprotein levels.

Bone resorption was determined by urinary N-telopeptide of type 1 collagen (uNTx) excretion rates. The 26 MGUS patients in this study had a mean uNTx level of 27.3 nmol/mmol creatinine at baseline with no patient having a baseline value above the upper range of normal. Nonetheless, a reduction in uNTx was noted in 7 out of 26 patients when given curcuminoids. This response coincided with the decrease in serum paraprotein and occurred after only one week of therapy.

The presented pilot study suggests that curcuminoids may decrease both serum paraprotein (in patients with levels ≥ 20 g/L) and uNTx in patients with MGUS. The potential role of curcuminoids as a therapeutic intervention for MGUS patients warrants further investigation. A double-blind, randomized control trial using higher dosages of curcuminoids in a larger cohort of MGUS patients with significant paraproteinaemia is planned next. Also, a more detailed study is required to assess the therapeutic role of curcuminoids in MGUS patients with elevated uNTx values.

Clinical Trials in Leukemia Predisposing Myelodysplastic Syndrome

The myelodysplastic syndrome (MDS), sometimes referred to as preleukemia, is a hematological condition characterized by ineffective production, or dysplasia, of myeloid blood cells and risk of transformation to acute myelogenous leukemia (AML). The anemia of MDS requires blood transfusions.

Potential of curcuminoids and ginger therapy in MDS patients was evaluated in 2006 at Saint Vincent's Comprehensive Cancer Center, New York, and University of Massachusetts Medical Center, Worcester (18). Management of lower-risk MDS patients who are still transfusion independent has not been extensively examined, being typically withheld until patients either become transfusion dependent or show signs of disease progression. Low-intensity therapies could be tried in these patients for two potential benefits: to improve the cytopenias and to determine whether intervention at this early stage could arrest or reverse the expansion of the abnormal cell clone. In the clinical trial reported here, curcuminoids

were combined with extract of ginger root (*Zingiber officinale* Roscoe, Fam. *Zingiberaceae*) standardized for gingerol (5% gingerol), the active constituent of ginger, to treat nine patients with low risk of myelodysplasia. Curcuminoids were administered in an incremental dosing schedule starting with 2.0 g in four divided doses per day increasing to 8 g per day, ginger extract 350 mg bid increasing to 1.4 g per day. The high dose of curcuminoids and ginger extract has been well tolerated with few or no side effects. The preliminary results have been very encouraging. Patients have been followed for 4 to 18 months and included seven males and two females: six presenting with refractory anemia (RA), two with refractory anemia with ringed sideroblasts (RARS), and one unclassified. Of the six evaluable patients, four have shown overall hematologic improvement. Two patients had stable disease. In conclusion, the curcuminoid and ginger extract combined therapy is well tolerated and potentially beneficial for early-stage MDS patients who are not transfusion dependent.

Clinical Trial in psoriasis vulgaris

Based on the physiological effects of curcumin and the positive anecdotal reports of its benefit for psoriasis, an open-label clinical trial to assess the safety and efficacy of oral curcuminoids in the treatment of chronic *psoriasis vulgaris* was conducted in the Department of Dermatology at the University of Pennsylvania School of Medicine and the Department of Dermatology at the University of Rochester, Rochester, New York (42).

Psoriasis is a chronic inflammatory skin condition classified as an autoimmune disorder which affects approximately 1% to 3% of the population worldwide and about 5.5 million people in the United States. There is a need for safe, inexpensive, and effective psoriasis therapies, especially because 95% of patients are willing to try new treatments. Curcuminoids have been used successfully to treat psoriasis based on anecdotal reports. A strong scientific rationale suggests that curcuminoids may in fact be promising for the treatment of psoriasis. In vitro and animal studies have demonstrated the inhibitory effect of curcuminoids on immune pathways critical to the pathology of psoriasis such as NFκB and downstream, inflammatory gene products such as Th-1–type cytokines (i.e., TNF-α, IFN γ,).

Design of the Trial

Patients were eligible if they were at least 18 years of age and had active but clinically stable plaque psoriasis that involved at least 6% of the body surface area and was of moderate plaque thickness as defined by a thickness score of 2 on the Psoriasis Area Severity Index (PASI). Patients were included if they were using a medically acceptable method of contraception throughout the entire study period. Patients with guttae, erythrodermic, or pustular psoriasis were excluded, as were patients who used systemic treatments for psoriasis (including methotrexate, cyclosporine, alefacept, adalimumab, efalizumab, infliximab, etanercept, etretinate, systemic steroids, and PUVA) within three months prior to day 0 or at any time during the study. Patients were excluded if they had used topical treatments or phototherapy for their psoriasis within 14 days prior to day 0 or at any time during the study. Patients who were pregnant or nursing a child, had clinically significant laboratory abnormalities at screening, or had significant uncontrolled comorbidities were excluded from the study. Subjects for whom the dose of clonidine, digoxin, beta-blockers, lithium, or antimalarials had changed in the past month prior to enrollment were excluded from the study. Enrolled subjects were required to avoid prolonged exposure to sun or UV light and to

discontinue nonmedicated emollients and medicated psoriasis shampoos 24 hours before each study visit.

The study was designed to determine the safety and efficacy of oral curcuminoids in patients with plaque psoriasis receiving 4.5 g per day of oral curcuminoids for the first 12 weeks followed by a 4-week observation period after discontinuing the study drug. End points included improvement in Physicians Global Assessment (PGA) score, PASI score, and safety end points throughout the study.

The primary measure of efficacy was the proportion of patients who were classified as a responder using the PGA of Change at week 12. A responder was defined as achieving a rating of good (50%–74% improvement), excellent (75%–99% improvement), or cleared (100% improvement) on the PGA compared to baseline. Secondary end points included PASI scores and health-related quality of life as measured by the Skindex-29. Other outcome measures include PASI 75 and PASI 50, which correspond to 75% and 50% improvements in PASI scores from baseline, respectively, and have been shown to represent a meaningful end point in psoriasis clinical trials. Subjects were classified as responders based on week 12 PGA scores and a response rate was calculated with 95% exact confidence intervals.

Median PASI and Skindex-29 scores were calculated at baseline and week 12. The 12 subjects were enrolled and received the investigational drug at day 0. Of the five subjects who did not receive the drug, three were excluded because they did not have ≥6% of their body surface area covered with psoriasis and another subject was excluded because of anemia. Subjects were instructed to take three capsules (each capsule containing 500 mg curcuminoids) three times per day. All subjects who completed the trial were at least 85% compliant with the treatment regimen as determined by patient diaries, patient interviews, and pill counts.

Tolerance and Side Effects

There were no study-related adverse events that necessitated participant withdrawal. The 4.5 g per day for 12 weeks of oral curcuminoids was well tolerated and safe in subjects with psoriasis. All adverse events possibly related to the study drug were mild and limited to gastrointestinal upset and heat intolerance or hot flashes. One subject who was eligible for the study was lost to follow-up before receiving any study drug. Eight subjects completed the trial up to week 16. Four of the 12 enrolled subjects did not complete the trial; one was withdrawn by the investigators due to worsening of her psoriasis and three withdrew prior to week 12 because of lack of efficacy. Subjects who failed to complete the trial had similar degrees of psoriasis severity as measured by PASI compared to patients who completed the trial, but noncompleters had more impairment in health-related quality of life at baseline as measured by Skindex-29 ($P = 0.04$).

Clinical Status

Descriptive statistics and baseline data for enrolled subjects are summarized in Table 6.3. Intention-to-treat analysis, in which all subjects who withdrew from the trial were classified as nonresponders, showed a response rate of 16.7% (95% CI: 2%, 48%). The secondary as treated analysis, which included only subjects who competed the trial up to week 12, had a response rate of 25% (95% CI: 3%, 65%). Only two subjects who completed the trial achieved a response at week 12. The two subjects who were classified as responders achieved

Table 6.3. Psoriasis Trial: Baseline Demographic and Clinical Information

Subjects	*Age (Median, IQR)*	*Sex (N, %) Male*	*Median (IQR)# of Prior Systemic Agents/Phototherapy Used*	*Race*	*Baseline PASI (Median, IQR)*	*Baseline Skindex (Median, IQR)*
Completed trial						
N=8	50.5 (45, 55)	N=7 (87.5%) male	1.5 (1, 2.5)	N=7 White N=1 Asian	13.7 (9.7, 17.2)	34.6 (18.5, 50.9)
Did not complete trial						
N=4	50 (38.5, 62.5)	N=2 (50%) male	1.5 (0.5, 2.5)	N=2 White N=1 Black N=1 Other	14.6 (5.4, 8.3)	63.2 (52, 79.9)

Source: *J Am Acad Dermatol*. 2008 Apr;58(4):625–631.

a score of excellent on the PGA, were seen during the winter months, and were not exposed to sunlight during the trial based on patient report and physical examinations (Figure 6.6). No patients received a score of "cleared," "good," or "fair" at week 12, while two subjects received a PGA score of "slight," three of "unchanged," and one of "worse" at week 12. Both responders achieved a PASI 75 at week 12, while no other subjects achieved a PASI 75 or a PASI 50 (Table 6.4).

Four weeks after discontinuation of curcuminoid therapy, the responders maintained an excellent response based on PGA and PASI 75 at week 16. In those subjects who completed the trial, the median Skindex-29 score was reduced by 0.35 (IQR 5.5, 5.0) (a lower score signifies improvement in quality of life). In subgroup analysis, the two responders had a median reduction in Skindex-29 scores of 16 (IQR 5.1, 26.9) (n=2), whereas nonresponders had a median increase (worsening) in Skindex scores of 2.5 (IQR 0, 5.6) (n=6).

The efficacy of the study drug was low, with an intention-to-treat response rate of 16.7%. The confidence interval for the 16.7% response rate is wide because of the small sample size, and therefore the study cannot exclude the possibility that the response observed was caused by a placebo effect or the natural history of skin disease in these subjects rather than efficacy of the drug itself. The lack of any evidence of meaningful response (e.g., PASI 50) in all other subjects argues that the true response rate is very low, limited to a small subset of psoriasis patients, or not caused by curcuminoids but rather by other factors that cannot be accounted for in an uncontrolled study. Nevertheless, excellent responses were observed in two patients, and therefore, large, placebo-controlled trials will be necessary to definitively prove or disprove oral curcuminoids as a potential therapeutic agent for psoriasis.

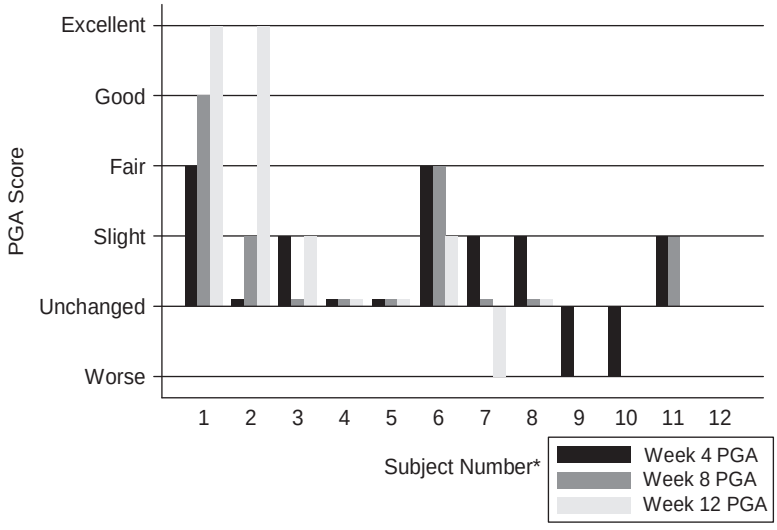


Figure 6.6. Psoriasis Trial: PGA Scores by Month

Week 12 PGA scores of "Good," "Excellent," and "Cleared" are classified as responders. Subjects 9–12 withdrew prior to week 12 and PGA scores, when available, are shown for these subjects for the weeks in which they were evaluated.

Source: *J Am Acad Dermatol*. 2008 Apr;58(4):625–631.

Table 6.4. Psoriasis Trial: Summary of Efficacy Endpoints at Week 12

Efficacy Outcome	*Results of Subjects Who Completed Trial (n=8)*	*Results of Intention to Treat Analysis (n=12)**
Response rate based on achieving at least a PGA of "good"	25%, 95% CI (3%, 65%)	16.7%, 95% CI (2%, 48%)
PGA at week 12 (median, IQR)	Unchanged—slight improvement (unchanged, fair-good)	Unchanged (worse-unchanged, slight improvement)
PASI 75 at week 12	25%, 95% CI (3%, 65%)	16.7%, 95% CI (2%, 48%)
Change in PASI (baseline-week 12) (median, IQR)	5.4 (0.65, 7.6) p=0.04	0.65 (–1.25, 6.5) p=0.26
Change in Skindex** (median, IQR)	0.35 (IQR –5.5, 5.0) p=0.9	0.0 (–2.55, 4.95) p=0.63

*Change in PASI data of 2 of 4 subjects who withdrew are available (–11.1 and –4.1), whereas the other two subjects who withdrew had no follow-up PASI data and were given a score of zero to indicate no change.
Change in Skindex data was only available for 1 of 4 subjects who withdrew and was 8.4. The other three subjects were given a score of zero to indicate no change.

**positive values indicate an increase in Skindex scores, suggesting a decrease in QOL.

Source: *J Am Acad Dermatol*. 2008 Apr;58(4):625–631.

CURCUMINOIDS IN PREVENTION AND TREATMENT OF NEURODEGENERATIVE CONDITIONS

Curcuminoids in Alzheimer's Disease: Ex Vivo Study

In 2004–2005, the Department of Medicine, Greater LA VA Medical Center and UCLA School of Medicine, Los Angeles, California, started testing a hypothesis that curcuminoids, which have epidemiologic and experimental rationale for use in Alzheimer's disease, may improve the innate immune system and increase amyloid clearance from the brain of patients with sporadic Alzheimer's disease (9, 10).

One of the most challenging fields in antiaging medicine is the management and treatment of chronic degenerative conditions as exemplified by Alzheimer's disease. This disease is increasingly seen as a defective response of the aging immune system to wear-and-tear damage and inflammation that occur with aging of the organism.

The aging immune system becomes progressively less efficient in dealing with inflammation. The innate and adaptive (acquired during lifetime) immune responses show age-related changes that could be decisive for healthy aging and survival. Natural or innate immunity is particularly important in the aging process and is based on macrophages, which are crucial for antimicrobial defense and removal of cellular and metabolic debris. Innate immunity functions result from a macrophage's ability to recognize a pattern of a pathogenic molecule through a code system called pathogen-associated molecular patterns (PAMPs). These potentially harmful molecules, for example, amyloid protein, when recognized by macrophages, trigger responses that also guide an appropriate adaptive immune response. The interaction between the innate and adaptive immune systems is critical for the clinical outcome of a pathogen molecule challenge to an organism—a difference between macrophages contributing to the body injury or to the healing process.

There is increasing evidence supporting a role for macrophages and the dependent innate immunity in Alzheimer's disease origins and progression. Brain amyloidosis is hypothesized to be a crucial pathogenic mechanism in the brain of a person with Alzheimer's disease, and many investigators of Alzheimer's disease pathogenesis believe that accumulation of amyloid-β (Aβ) is toxic to neurons. The immune system of patients with Alzheimer's disease is generally poorly responsive to Aβ and unable to remove amyloid from neurons. The amyloid hypothesis of Alzheimer's disease has increased interest in developing therapies that promote clearance of brain amyloidosis by macrophages leading to a novel strategy of immunotherapy with Aβ vaccine, or antibodies against the amyloid protein. It was established that the anti-Aβ antibodies were sufficient for reducing Aβ in the brain and that these reductions were accompanied by improvement in cognitive function in animal models of Alzheimer's disease.

The ex vivo study reported here was performed on peripheral blood mononuclear cells (PBMC) obtained from patients with Alzheimer's disease and healthy controls. All subjects gave informed consent approved by the UCLA Institutional Review Board for Human Studies. The diagnostic criteria for Alzheimer's disease satisfied the National Institute of Neurological and Communicative Disorders and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria for probable Alzheimer's disease as described. Normal age-matched control subjects were recruited from UCLA faculty and

alumni. Peripheral blood mononuclear cells from six patients were separated from EDTA-anticoagulated blood by centrifugation on Ficoll-Hypaque gradient. Differentiated macrophages were treated with curcuminoids (0.1 *m*M) in the medium overnight and were then exposed to beta amyloid Aβ conjugated with fluorescein isothiocyanate (FITC) (FITC-Aβ) and examined by fluorescence or confocal microscopy.

Macrophages of a majority of patients with Alzheimer's disease did not phagocytose and did not efficiently clear amyloid from the brain, although they were able to phagocytose bacteria. In contrast, macrophages of normal subjects phagocytosed amyloid. Upon amyloid stimulation, macrophages of normal subjects accelerate synthesis of molecules and receptors that participate in the system of pathogen recognition, specifically, MGAT3 (beta-1,4-mannosyl-glycoprotein 4-N-acetylglucosaminyltransferase) and Toll-like receptors (TLRs), whereas mononuclear cells of patients with Alzheimer's disease generally down-regulate these genes. Defective phagocytosis of the amyloid may be related to suppression of these pathogen recognition molecules. In mononuclear, macrophage-like cells isolated from peripheral blood in patients with Alzheimer's disease, curcuminoids, especially bisdemethoxycurcumin, may enhance defective phagocytosis of amyloid (see Figure 6.7) while restoring synthesis critical for phagocytic function molecules and receptors, MGAT3 and TLRs (9, 10). Therefore, curcuminoids may provide a novel approach to Alzheimer's disease immunotherapy that is safer than the recently suggested vaccine therapy.

The salient result of the current study is that macrophages of three patients, 50% of those tested, showed a significant increase in total Aβ uptake after curcuminoid treatment in vitro. The responding patients were younger and had a higher Mini Mental State Exam (MMSE) score, suggesting that patients in a less advanced stage of Alzheimer's disease may respond better. Further studies are needed to resolve the factors determining good response to curcuminoids. In vitro testing of curcuminoids in macrophage cultures may be useful in individualizing the treatment of patients with Alzheimer's disease. Testing Aβ phagocytosis in Alzheimer's disease macrophages might be helpful for assessing the ability of patients to respond to immunomodulatory therapy with curcuminoids.

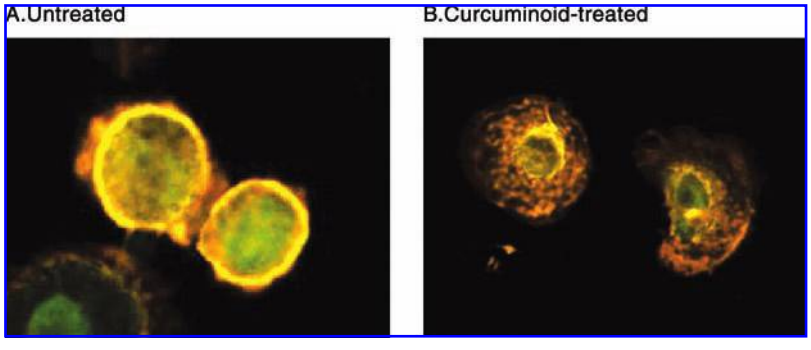


Figure 6.7. Alzheimer's Disease *ex Vivo*: Phagocytosis of Ab by AD Macrophages Is Increased by Curcuminoid Treatment

Confocal microscopy, FITC-Ab (green), phalloidin-FITC (red), colocalization (yellow). Note surface binding in (A) vs. intracellular uptake in (B).

Source: *J Alzheimers Dis.* 2006 Sep;10(1):1–7.

Curcuminoids in Alzheimer's Disease: Preliminary Clinical Study

In 2004–2008, a preliminary clinical trial of curcuminoids in Alzheimer's patients was conducted at the Mary S. Easton Center for Alzheimer's Disease Research in the UCLA Department of Neurology (22). The main objective of the study was to obtain tolerability and preliminary efficacy data of 2 g per day and 4 g per day of curcuminoids in mild to moderate Alzheimer's disease patients in a 24-week, randomized, double-blind study.

The group of 36 patients with Alzheimer's disease was evenly divided and randomized to receive placebo, 2 g, or 4 g per day of curcuminoids in two divided doses. The MMSE, ADAS-Cog (Alzheimer's Disease Assessment Scale-Cognitive subscale), NPI (Neuropsychiatric Inventory), and ADCS-ADL (Alzheimer's Disease Cooperative Study-Activities of Daily) scales were performed at baseline and at 24 weeks' time. Plasma samples and lumbar punctures were also performed at baseline and 24 weeks and Abeta40, Abeta42 measured in plasma and cerebrospinal fluid (CSF) and total tau and p-tau measured in CSF. Baseline and 24-week values were compared between groups using repeated measures ANOVA. Adverse events were recorded at each visit.

A total of 11 patients on placebo, 9 patients on 2 g of curcuminoids, and 10 patients on 4 g of curcuminoids completed the study. In an analysis of completers, there were no significant differences or favorable trends between placebo and treatment groups on clinical measures. No difference in plasma or CSF biomarkers were found between groups except for a tendency toward a lesser degree of increase in plasma Abeta40 in the combined group on 2 and 4 g per day of curcuminoids.

The curcuminoids were well tolerated except for some suggestions of gastrointestinal side effects in the form of diarrhea at both dose levels by the patients. However, this preliminary clinical trial does not support a large effect of orally administered curcuminoids on clinical and biomarker measures in patients with mild to moderate Alzheimer's disease at doses of 2 to 4 g per day. Limitations to the interpretation of this study include the small sample size, limited time of administration especially in view of interesting data on Abeta40 plasma levels, effective dose, and uncertain bioavailability of curcuminoids.

The interesting immunological mechanism of curcuminoids in Alzheimer's disease and safety of its clinical use calls for larger dose escalation clinical studies of longer duration in a population of patients who have mild to moderate Alzheimer's disease.

CURCUMINOIDS OVERVIEW OF CLINICAL USE, SAFETY, AND PHARMACOLOGY

The clinically effective and safe dose of curcuminoids and their form and route of administration to the body are the subject of ongoing controversy. In fact, mundane technical aspects of curcuminoid administration may separate success from failure in the therapy with these compounds.

In India and Ayurvedic traditions, turmeric is ingested with milk, especially when boiled with milk, and applied topically or ingested with oil preparations, for example, coconut oil, almond oil, mustard oil, or sesame oil. Curcuminoids are poorly absorbed from the gastrointestinal tract, with low nanogram levels of circulating curcuminoids detected in the plasma (43). Current clinical experience indicates that oral supplementation of curcuminoids is

tolerated without toxicity at doses of up to 8 g daily for up to 18 months and that this dose may result in therapeutic activity in some patients with cancer (7, 16, 18, 19).

Dose Escalation Study

Dose escalation study was performed at the Division of Hematology-Oncology, Department of Internal Medicine, University of Michigan, to determine the maximum tolerated dose, safety profile, and resultant plasma concentration of a single dose of curcuminoids (43). The study protocol and the comprehensive written informed consent used in this study protocol were reviewed and approved by the University of Michigan Human Subject Review Board prior to the start of the study. Eligible participants were healthy male and female volunteers, 18 years of age or older, who had not consumed any curry- or curcumin-rich foods to their knowledge within the previous 14 days. Thirteen men and eleven women with mean age of 34 years (range: 19–74 years) were enrolled in the study. The racial distribution was 18 Caucasians and 6 African Americans.

After written informed consent was obtained, three subjects were entered consecutively at dose levels of 500, 1000, 2000, 4000, 6000, 8000, 10000, and 12000 mg. Subjects took the dose with 8 fl. oz. of water followed by a standard meal containing dietary fat (providing 34 g or 42 g fat, per 2200 kcal/day or 2500 kcal/day meal plan, respectively).

Safety was assessed for 72 hours following the curcuminoid dose. Toxicities were graded based on National Cancer Institute Common Toxicity Criteria version 2.0. The maximum tolerated dose was the highest dose that did not cause escalation to cease.

Blood specimens from all subjects on the escalation phase were obtained just prior to dosing and one, two, and four hours after completing dosing at each dose level tested.

Seven adverse events occurred, all were reported as grade 1, and none appeared to be dose related (Table 6.5). No curcumin was detected in serum of participants administered 500, 1000, 2000, 4000, 6000, or 8000 mg of curcuminoids. In two subjects taking 10000 mg and 12000 mg curcuminoids, respectively, plasma curcumin was detectable: 30.4 ng/ml after one hour (10000 mg), 39.5 after two hours (10000 mg), 50.5 after four hours (10000 mg), 29.7 after one hour (12000 mg), 57.6 after two hours (12000 mg), 51.2 after four hours (12000 mg) (Table 6.6). No plasma concentrations of curcumin were detected in the remaining subjects at the 10000 or 12000 mg dose levels.

Table 6.5. Dose Escalation: All Adverse Events by Dose Levels

Dose Level[a]	*Type*	*No. of Events*	*Toxicity Grade*[b]
1000 mg	Diarrhea	1	1
4000 mg	Headache	1	1
8000 mg	Rash	1	1
	Yellow stool	1	1
10000 mg	Yellow stool	1	1
	Headache	1	1
12000 mg	Diarrhea	1	1

Source: *BMC Complement Altern Med*. 2006; Mar 17; 6–10.
[a]Total of 3 subjects at each level.
[b]National Cancer Institute. Common Toxicity Criteria v.2.0.

Table 6.6. Dose Escalation: Serum Curcumin Levels in Mg/Ml for Two Subjects

Dose	*Baseline*	*One Hour*	*Two Hour*	*Four Hour*
10000 mg	Approx .6.0	30.4	39.5	50.5
12000 mg	Approx trace	29.7	57.6	51.2

Source: *BMC Complement Altern Med*. 2006; Mar 17; 6–10.

Dose and Form of Curcuminoids in Relation to Efficacy

Reported low blood levels of curcumin may be caused in part by the extensive intestinal and hepatic metabolic biotransformation of curcuminoids. Preclinical and clinical work has demonstrated that avid sulfation, glucuronidation, and reduction of curcumin occur in the gastrointestinal tracts of rats and humans (45, 46, 47). Animal experiments suggest that 65% to 85% of orally administered curcuminoids are excreted unchanged in stool with negligible amounts excreted in the urine (48).

Interestingly, plasma concentrations of curcumin released from conjugated forms are surprisingly low, and there is little evidence for the biological activity of curcumin conjugates against malignant cell growth (45). Possibly there are other forms of conjugated curcumin or derivatives of curcuminoids that can better explain their biological activity and provide the future formulae for more effective clinical application. In an in vitro study, the anti-inflammatory and anticancer properties of curcumin (Cur) and its derivatives demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) were evaluated and compared. The results indicate that the relative potency for suppression of tumor necrosis factor was Cur>DMC>BDMC, thus suggesting the critical role of methoxy groups on the phenyl ring in a biological potential of curcuminoids (49).

It is also possible that curcuminoids may exert different biological properties at different doses in specific clinical conditions. In an experiment on prevention of Alzheimer-like pathology in mice, the effect of a low (160 ppm) and a high (5000 ppm) dose of dietary curcumin on inflammation, oxidative damage, and plaque pathology was evaluated. Low and high doses significantly lowered oxidized proteins and IL-1B, a proinflammatory cytokine usually elevated in the brains of these mice. With low-dose, but not high-dose, curcumin treatment, the astrocytic marker glial fibrillary acidic protein was reduced, and insoluble beta-amyloid ($A\beta$), soluble $A\beta$, and plaque burden were significantly decreased, by 43% to 50% (50).

Despite the discussed complexities of mechanism of action, the biological activity of curcuminoids is beyond question, with biomarkers of inflammation, for example, NF-κB and COX-2, suppressed by oral administration of curcuminoids with clinical improvement in the treated conditions (7, 16, 17, 18, 19, 20).

The above-presented clinical studies, for example, curcuminoids employed in pancreatic cancer patients, indicate that despite low levels of curcumin detectable in plasma (e.g., 22–41 ng/mL), some of the patients improved as evidenced by significantly lowered cytokine levels (NF-kappaB, COX-2, and pSTAT3) and shrinking of the tumor mass. Conceivably, the limited bioavailability of curcumin attenuated the response rate, because exposure to microgram amounts of curcumin is required to show antiproliferative effects in vitro. It should also be taken under consideration that circulating curcumin

levels do not reflect tumor tissue curcumin levels. In another human trial, the glutathione *S*-transferase activity, a potential surrogate biomarker of curcumin activity, was significantly decreased in patients taking 440 mg curcuminoids per day despite the lack of measurable blood levels of curcumin (46). This finding suggests that the metabolites of curcumin and its derivatives, which may not have been detected, resulted in a systemic biological effect.

Route of Administration in Relation to Efficacy

The form and route of administration of curcuminoids are important aspects for future chemopreventive strategies with these compounds. Curcumin and its derivatives are hydrophobic and therefore cannot be given intravenously. However, because curcumin and its derivatives are lipophilic, they can be encapsulated in a liposome, and this preparation would allow IV administration, possibly leading to higher circulating levels of curcumin. It has been reported previously that liposomal curcumin has antitumor activity both in vitro and in vivo and has no overt toxicity in animal models when administered systemically (44). Therefore, development of liposomal curcuminoids for clinical trials in cancer patients is a worthwhile strategy. Another possibility under consideration involves a nanoemulsion of curcuminoids to bypass the gastrointestinal barrier to achieve higher plasma concentrations of the unaltered phenolic compounds (51).

Curcuminoids may have efficacy when applied topically as an anti-inflammatory, antioxidant, and anticancer preparation. One study evaluated topical gel delivery of curcuminoids for its anti-inflammatory effects (52). Carbopol 934P (CRB) and hydroxypropylcellulose (HPC) were used for the preparation of gels. The percutaneous flux and enhancement ratio of curcumin across rat epidermis was enhanced significantly by the addition of menthol to both types of gel formulations. Neither type of topical gel formulation, CRB or HPC, caused any skin irritation. CRB gel showed better anti-inflammatory effects in vivo as compared to HPC gel. Both formulations were comparable in their topical anti-inflammatory action to a standard diclofenac gel formulation.

Absorption- and Bioavailability-Enhancing Compounds

Administering curcuminoids combined with agents enhancing their absorption may result in better biological efficacy of these compounds. In a trial performed at the Department of Pharmacology, St. John's Medical College, Bangalore, India, the effect of piperine extracted from fruits of black pepper (standardized for a minimum 95% alkaloid piperine) on gastrointestinal absorption of curcuminoids was evaluated (53).

Ten healthy male volunteers, 20 to 26 years old and weighing 50 to 75 kg, participated in a randomized trial to determine the comparative bioavailability and pharmacokinetic profile of curcuminoids when given alone or with piperine. Complete physical examination and blood work were performed to confirm that the subjects were in good health when enrolled into the study. The study protocol was reviewed and approved by the Institutional Ethical Committee, and informed consent was obtained from all subjects.

Participants abstained from food from 10 PM of the previous evening and reported to the laboratory at 7AM the following day. The blood samples were collected at times 0 (predrug

time) and 25, 30, and 45 minutes and 1, 2, 3, 4, and 5 hours after intake of curcuminoids. Following the predrug time (time 0) blood sample collection, the dose of 2 g curcuminoids alone (four capsules each containing 500 mg of curcuminoids) or 2 g curcuminoids with 20 mg piperine (four capsules each containing 5 mg of piperine) were ingested by the participants of the study. The blood sampling and oral curcuminoids were repeated after two weeks of washout. The subjects were asked to refrain from smoking, drinking alcohol, and taking any drugs during the trial. Standard meals were given to all participants on the day of the test.

Curcuminoids alone or in combination with piperine were well tolerated by the volunteers with no subjective or objective side effects reported. Serum levels of curcumin when administered alone were very low at all time points in most of the subjects. However, when piperine was included in the formula, serum concentrations of curcumin were significantly increased at 25 minutes and 30 minutes ($p < 0.01$) and 45 minutes ($p < 0.001$) of blood testing time points. Subsequently, there was a rapid decline of serum levels of curcumin within the next one hour and gradual decline to undetectable levels by the three-hour time point. The pharmacokinetic parameters showed the mean Cmax of curcumin at 0.006 ug/ml when curcuminoids were administered alone, versus 0.18 ug/ml when curcuminoids were administered with piperine. The mean AUC (area under the curve) with curcuminoids administered alone was 0.004 ug/h/ml versus 0.08 ug/h/ml when curcuminoids were administered with piperine. Therefore, the relative bioavailability of curcuminoids when given with piperine was enhanced by 2000% as compared to values of curcuminoids alone.

The results of this study indicate that piperine may operate through any or all of the following mechanisms: (a) a nonspecific enhancement of gastrointestinal absorption, (b) a potent inhibition of drug metabolism via P450 enzymes, and (c) inhibition of glucuronidation altering the disposition of the drug. The postulated biological mechanisms suggest that piperine may be involved in inhibiting the metabolism of curcuminoids and enhancing their bioavailability. Piperine may enhance serum concentration of curcuminoids probably because of increased absorption, reduced metabolism, and decreased rate of elimination from the body. Therefore, piperine may be considered as a bioenhancer of curcuminoids administered orally. Because of its lipophylic nature, piperine may be considered in future parenteral formulations encased in liposomes with curcuminoids.

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