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Review

Chios Mastic Gum: A Plant-produced Resin Exhibiting Numerous Diverse Pharmaceutical and Biomedical Properties

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Abstract. *Chios mastic gum (CMG) is a resin produced by the plant Pistacia lentiscus var. chia. CMG is used to extract the mastic gum essential oil (MGO). CMG and MGO consist of nearly 70 constituents and have demonstrated numerous and diverse biomedical and pharmacological properties including (a) eradication of bacteria and fungi that may cause peptic ulcers, tooth plaque formation and malodor of the mouth and saliva; (b) amelioration or dramatic reduction of symptoms of autoimmune diseases by inhibiting production of pro-inflammatory substances by activated macrophages, production of cytokines by peripheral blood mononuclear cells in patients with active Crohn's disease, and suppression of production of inflammatory cytokines and chemokines in an asthma model in mice; (c) protection of the cardiovascular system by effectively lowering the levels of total serum cholesterol, low-density lipoprotein and triglycerides in rats, and protection of low-density lipoprotein from oxidation in humans; (d) induction of apoptosis in human cancer cells in vitro and extensive inhibition of growth of human tumors xenografted in immunodeficient mice; and (e) improvement of symptoms in patients with functional dyspepsia. Collectively taken, these numerous and diverse medical and pharmaceutical properties of CMG and MGO warrant further research in an effort to enhance specific properties and identify specific constituent(s) that might be associated with each property.*

Chios mastic gum (CMG) is a resin produced by the plant *Pistacia lentiscus* var. chia, which is cultivated in countries of the Mediterranean sea and particularly in the southern part of the Greek island of Chios. Interestingly, the *P. lentiscus* plant can be planted or re-planted in other areas of the world, including the northern section of Chios island, but it will not produce the resin. The oldest references to mastic have been traced back to Herodotes in the 5th Century B.C.. Since 3000 B.C., Greeks have used CMG in diverse applications, such as cooking, preparation of beverages, cosmetics and paints, and for treatment of gastric ailments. Since 1997 it has been characterized as a product of Protected Designation of Origin by the European Union. CMG has played an important role in the economy of the island of Chios during various periods of Greek history, ancient times, the years of the Ottoman occupation of Chios, as well as the present time. CMG-derived products are thus considered materials of ethnic importance, and very likely materials for the discovery of novel pharmaceutical agents for the treatment of numerous and diverse human and animal diseases. On this regard, researchers have only just started to associate specific pharmaceutical properties of CMG with specific components.

CMG forms as a resin of teardrop-like appearance on the stem and branches of the plant at sites of incisions created with a special tool. Initially, CMG is secreted as a colloidal clear liquid that solidifies into various shapes after 15-20 days. Solidified CMG has a crystalline appearance of a stalactite and drops to the ground under the tree. The material on the ground is collected by hand and further processed for packing and use. The hardness of the CMG depends on the atmospheric temperature, exposure time in nature, and the size of the tear-like product. For more information about *Pistacia lentiscus* var. *chia* and CMG, see www.gummastic.gr, www.drhoffman.com and en.wikipedia.org/wiki/*Pistacia*_lentiscus.

CMG is highly insoluble in water, but is somewhat soluble in different organic solvents, including methanol, dimethylsulfoxide (DMSO), acetone and chloroform. The chemical composition of various extracts from CMG, CMG-derived mastic gum oil (MGO), and the insect galls on *P. lentiscus* have been analyzed, and several constituents have been isolated and identified in various fractions (1-7). In

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general, out of the 69 constituents of MGO, 61 have been identified. Six components, namely α-pipene, β-pipene, β-myrcine, linalool, *trans*-caryophyllene and camphene, comprise 65% to 80% of the weight of the extracted products. The same major components were found in CMG, which proved to be more difficult to handle than MGO (3, 5).

In this review, major publications describing pharmaceutical and medicinal properties of CMG and its various extracts have been summarized.

Antibacterial Properties

In this section, we report results of studies on the effects of CMG and GMO on bacteria that have been proven, or considered to be the causative agents of various diseases.

Studies of duodenal ulcer. In the 1980s, it was first reported that CMG was a potential agent for the treatment of duodenal ulcer in humans (7). A double-blind clinical trial was carried out on 38 patients with symptomatic and endoscopically proven duodenal ulcer to compare the therapeutic responses to mastic (1 g daily, 20 patients) and placebo (lactose, 1 g daily, 18 patients) given orally over a period of two weeks. Symptomatic relief was obtained in 16 (80%) patients on CMG and in 9 (50%) patients on placebo. In addition, endoscopically-proven healing occurred in 14 (70%) patients on CMG and 4 (22%) patients on placebo. The differences between treatments were highly significant. CMG was well-tolerated and did not produce side effects. It was concluded that CMG has a healing effect on ulcers (7). In another study, the effect of CMG on experimentally-induced gastric and duodenal ulcers in rats was evaluated (8). The reduction in the intensity of ulceration in cysteamine-induced duodenal ulcers was not found to be statistically significant in mastic-pre-treated rats. The results suggested that a mild antisecretory and localized adaptive cytoprotectant action may be responsible for the anti-ulcer activity of CMG. In a third study, the antibacterial activity of CMG was evaluated against clinical isolates of *Helicobacter pylori*, the bacterium that represents the major etiological agent of gastritis, gastric, and duodenal ulcer disease and can cause gastric cancer and mucosa-associated lymphoid tissue B-cell lymphoma (9). The influence of CMG on the morphology of *H. pylori* was determined by transmission electron microscopy. CMG was found to induce protrusions, morphological abnormalities, and cellular fragmentation in *H. pylori* cells, indicating that CMG exhibits anti-*H. pylori* activity (10). Finally, a recent report presents evidence that CMG inhibits *H. pylori* inflammation by inhibiting neutrophil activation *in vitro* (11). These observations were confirmed by a study of the effect of CMG on *H. pylori* eradication in patients suffering from an *H. pylori* infection. Fifty-two patients were randomly divided into four groups and received treatment with low CMG dose (group A), high CMG dose (group B), pentoprazol plus low CMG dose (group C), for 14 days, and pentoprazol plus amoxicillin plus clarithromycin (group D) for 10 days. All patients were positive for *H. pylori* prior to entering the study, as detected by a urea breath test (UBT). Eradication of *H. pylori* was demonstrated in 4/13 patients of group A and in 5/13 patients of Group B, whereas eradication was not observed in all patients of group C and in 10/13 patients of group D were negative, as shown by the UBT. All patients tolerated mastic gum well while the mild side effects were reversible. It was concluded that CMG has bactericidal activity against *H. pylori in vivo* (12). A recent report claimed that extracts and pure major constituents of CMG were active against *H. pylori* (6). In that study, a total mastic extract without polymer (TMEWP) was prepared after the insoluble polymer was removed in order to improve solubility and enhance *in vivo* activity. After chromatographic separation, the acid fraction yielded the major triterpenic acids, while the neutral fraction yielded several triterpenic alcohols and aldehydes. CMG extracts and isolated pure triterpenic acids were tested for *in vitro* activity against a panel of 11 *H. pylori* clinical strains. The acid fraction was found to be the most active extract; the most active pure compound in that fraction was isomasticadienolic acid. The results showed that administration of TMEWP may be effective in reducing *H. pylori* colonization and that the major triterpenic acids present in the acid extract may be responsible for such an activity (6).

Although the results described so far support an anti-*H. pylori* activity of CMG, there are also reports that CMG has no effect on *H. pylori* (13, 14). In one study, CMG monotherapy was used to determine its ability to eradicate *H. pylori* infection in mice. Mice were inoculated intragastrically with either a suspension of *H. pylori* or brain-heart infusion broth alone, which served as negative control. Subsequently, the mice were given 2 g of mastic twice daily for 7 days four weeks after infection as an antimicrobial chemotherapy. The results demonstrated the inability of CMG to eradicate *H. pylori* infection in mice. It has also been reported that CMG failed to suppress or eradicate *H. pylori* infection in humans. In that study, nine patients with *H. pylori* infection, and without gastroduodenal ulceration, were recruited from day-case endoscopy lists and treated with 1 g of CMG four times daily for 14 days. CMG had no effect on the *H. pylori* status in any of the eight patients who completed the study; all remained *H. pylori*-positive. Thus, it was concluded that despite the reported anti-*H. pylori* action *in vitro*, CMG appears to have no effect on *H. pylori* in humans (13). This discrepancy in the reported findings cannot be explained. However, the majority and the most recent studies demonstrate that CMG can prevent and/or eliminate gastroduodenal ulcers.

Studies with various micro-organisms. This section includes major reports describing the ability of CMG to kill micro-

organisms other than *H. pylori*. On this regard, an early study was carried out to determine the *in vitro* antimicrobial activity of three essential oils and CMG against six bacteria and three fungi (3). In that study, *trans*-caryophyllene was found to be the major component by weight. Several trace components that appear to contribute significantly to the antibacterial activity of mastic oil were identified: verbenone, α-terpineol, and linalool. The sensitivity to these compounds varied for the different bacteria tested (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*). It was suggested that the efficacy of mastic oil is due to a number of its components working synergistically.

In another study, solid and liquid types of mastic had selective antibacterial action against oral bacteria *Porphyromonas gingivalis* and *Prevotella melaninogenica*, but no anti-HIV activity. CMG exhibited hydroxyl radical-scavenging activity. The selective antibacterial activity of CMG suggested its possible beneficial effects on oral health (15).

In general, chemical plaque control is a useful aid in mechanical oral hygiene, and various chemical agents have been evaluated for antiplaque activity. In this context, the antiplaque effect of chewing CMG was investigated. Twenty dental students who were both systemically and periodontally healthy participated in this study. The effects of CMG were assessed from two double-blinded, randomized studies. The total number of oral bacterial colonies was significantly reduced during the four hours of chewing CMG compared to the placebo gum. The mastic group had a significantly reduced plaque index and gingival index, compared to the placebo group. These results suggested that CMG is a useful antiplaque agent and that chewing CMG reduces both bacterial growth in saliva and plaque formation on teeth (16)

CMG is an ancient remedy for oral malodor, and this knowledge has been assessed in more recent studies. In one study, CMG eradicated *Porphyromonas gingivalis*, a known odorogenic periopathogenic oral bacterium, suggesting that CMG may be used as a potential non-toxic local agent in treating oral malodor and gum disease (17). In another study, it was determined that CMG exhibited antibacterial activity against *Streptococcus mutans* and *mutans streptococci* both *in vitro* and *in vivo* (18). In this study, saliva samples were obtained from the participants immediately before and after chewing either the CMG or the placebo (paraffin) gum for 15 min. Significantly fewer bacteria were found in saliva samples collected after chewing CMG than in those from the controls. This study showed that CMG has significant antibacterial activity against *S. mutans* and could be a useful adjunct in the prevention of caries (18). In a similar study the levels of *Streptococcus mutans*, lactobacilli, and total cultivated bacteria were measured before and after chewing CMG versus placebo gum. After chewing CMG for 15 min, a significant decrease in total bacteria and *S. mutans* was observed. The results showed that chewing CMG decreased the total number of viable bacteria, *S. mutans*, and lactobacilli in saliva in orthodontically-treated patients with fixed appliances. Therefore, chewing CMG might be useful in preventing caries (19).

Anti-inflammatory and Antioxidant Properties

In this section, we present studies that demonstrate the anti-inflammatory and antioxidant properties of CMG. On this respect, a recent review article categorized the natural triterpene congeners, which are involved in anti-inflammatory and anti-cancer effects on humans, according to their targets: phospholipase A_2 (PLA_2); cyclo-oxygenase (COX); and lipo-oxygenase (LOX) (20). Patients with chronic inflammatory diseases, such as cystic fibrosis, asthma, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and Crohn's disease (CD) have an increased atherosclerotic risk that cannot be explained by traditional cardiovascular risk factors alone. Recently, whether CMG can affect the function of activated macrophages was investigated (21, 22). The results showed that both solid and liquid CMG inhibited the production of pro-inflammatory substances such as nitric oxide (NO) and prostaglandin (PGE_2) by lipopolysaccharide (LPS)-activated mouse macrophage-like RAW264.7 cells (23). Western blot and (RT-PCR) analyses showed that CMG inhibited the expression of inducible NO synthase (iNOS) and COX-2 at both the mRNA and protein level. These data demonstrated that CMG inhibits the production of both NO and PGE_2 by activated macrophages mostly *via* its cytotoxic action (20). Furthermore, CMG inhibited protein kinase C, which attenuates production of H_2O_2 by NADPH oxidases (23), and carrageenan-induced statistically significant edema, supporting the suggestion that CMG could be used as an anti-inflammatory and antioxidant agent (22, 23)

Due to its anti-inflammatory properties, CMG could be used to reduce or ameliorate the symptoms in humans suffering from autoimmune diseases, such as CD. A study was conducted in patients with active CD, to assess the effects of CMG administration on cytokine production of circulating mononuclear cells. The study was conducted in patients with established mildly-to moderately-active CD who attended the outpatient clinics of the hospital and in healthy controls. Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), macrophage migration inhibitory factor (MIF), and intracellular antioxidant glutathione (GSH) were evaluated in peripheral blood mononuclear cells before and after CMG treatment. In patients with CD-CMG treatment resulted in the reduction of TNF-α secretion, while MIF release was significantly increased, indicating that the random migration and chemotaxis of monocytes/macrophages was inhibited. No significant changes were observed in IL-6, MCP-1, or GSH concentrations. These findings provide strong evidence that CMG might be an important regulator of immune function in CD (24).

The pathogenesis of allergic asthma is characterized by airway inflammation, eosinophilia and airway hyper-responsiveness (AHR). In one study, Qiao *et al*. (25) investigated the anti-inflammatory effects of mastic, obtained from the stem and the leaves of *Pistacia lentiscus* trees, on allergic asthma. In an ovalbumin-induced mouse asthma model, mastic significantly inhibited eosinophilia while reducing airway AHR and suppressing the production of inflammatory cytokines (IL-5 and IL-13), as well as chemokines eotaxin, eotaxin2 and (RANTES) in bronchoalveolar lavage fluid. Moreover, mastic potently inhibited eotaxin-induced eosinophil chemotaxis *in vitro,* without influencing eotaxin receptor-chemokine receptor 3 expression. These results suggest that CMG may contribute to the treatment of inflammatory diseases (25).

Heart-protective properties. Due to its anti-inflammatory and antioxidant properties CMG has been studied for its ability to modify molecules and complexes associated with heart function and specifically protection of the cardiovascular system. Under pathological conditions of hyperlipidemia, oxidative stress and/or genetic disorders, specific components of low-density lipoprotein (LDL) become oxidized, and the transport of cholesterol by modified LDL is diverted from its physiological targets toward excessive cholesterol accumulation in macrophages in the vascular wall. This pathological deposition of modified lipoproteins and the attendant pro-inflammatory reactions in the artery wall lead to the development of atherosclerotic lesions. Continued accumulation of immunogenic-modified lipoproteins and a pro-inflammatory milieu result in the progression of atherosclerotic lesions, which may obstruct the arterial lumen and eventually rupture and thrombose, causing myocardial infarction or stroke (26, 27). CMG protects the cardiovascular system by effectively lowering the levels of serum cholesterol and protects LDL from oxidation in humans. On this regard, attachment of leukocytes to the vascular endothelium and the subsequent migration of cells into the vessel wall are early events of atherogenesis, and this process requires the expression of endothelial adhesion molecules. However, data on the anti-inflammatory effects of CMG on endothelium are scarce. In a recent study, the effects of CMG and its constituent, tirucallol, were examined on the expression of adhesion molecules (VCAM-1) and (ICAM-1) and the attachment of monocytes (U937 cells) in TNF-α-stimulated human aortic endothelial cells (HAECs), by an adhesion assay. Both CMG and tirucallol significantly inhibited VCAM-1 and ICAM-1 expression in TNF-α-stimulated HAECs. They also significantly inhibited the binding of human leukemia U937 cells to TNF-α-stimulated HAECs and attenuated the phosphorylation of NF-κB p65. This study extends existing data regarding the cardioprotective effect of CMG, provides new insight into the mechanisms underlying the beneficial effect of CMG on endothelial function, and may aid in the design of new therapy for intervention in atherosclerosis (28).

In a related study, naturally-occurring gums and resins were tested for their possible protective effect against copper-induced LDL oxidation *in vitro* (29). CMG was the most effective substance in protecting human LDL from oxidation. The minimum and maximum doses for the saturation phenomena of inhibition of LDL oxidation were 2.5 mg and 50 mg CMG (75.3% and 99.9%, respectively). When fractionated in order to determine a structure-activity relationship, it was shown that the total MGO and acidic fractions of CMG exhibited a highly protective activity, ranging from 65.0% to 77.8% (29).

In a very recent study, we investigated the hypolipidemic effect of MGO in naïve and detergent-induced hyperlipidemia rats (30). MGO administration resulted in a dose-dependent reduction in the constitutive synthesis of serum cholesterol and triglycerides into naïve and hyperlipidemic rats. By testing various major components of MGO it was demonstrated that camphene is associated with the hypolipidemic action. The maximal beneficial dose of 30 μg camphene per gram of body weight in hyperlipidemic rats resulted in a 54.5% reduction of total cholesterol, 54% of LDL-cholesterol and 34.5% of triglycerides (30). Treatment of human hepatome HepG2 cells with camphene led to a decrease in cellular cholesterol content to the same extend as mevinolin, a known (HMG-CoA) reductase inhibitor. The hypolipidemic action of camphene is independent of HMG-CoA reductase activity, suggesting that its hypocholesterolemic and hypotriglyceridemic effects are associated with a mechanism of action different from that of statins.

Another study was carried out to assess the effects of CMG on cardiological and hepatic biochemical indices of humans (31). Participants were randomly assigned to two groups, the first ingesting 5 g of CMG powder in water, daily and the second receiving water alone (control). Serum biochemical parameters were determined on a monthly basis for 12-18 months. The group ingesting CMG exhibited a decrease in serum total cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein A-1, apolipoprotein B, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and γ-glutamyltransferase (γ-GT) levels; in the low-dose group, glucose levels decreased in males. These findings suggested that CMG could have a hepatoprotective/cardioprotective role *in vivo* in humans (31).

Anticancer activities. To investigate the modifying effects of CMG on rat liver carcinogenesis, rats were subjected to the established rat liver medium-term carcinogenesis bioassay. Initially, rats were intraperitoneally injected with 200 mg/kg body weight of diethylnitrosamine (DEN). The results showed that liver weights significantly increased in a CMG dose-

dependent manner, leading to the conclusion that CMG is potent of promoting formation of pre-neoplastic lesions in the established rat liver medium-term carcinogenesis bioassay (32).

Chronic inflammation has long been recognized as a risk factor for many tissues of human cancer. One mechanistic link between inflammation and cancer involves the generation of nitric oxide, superoxide, and other reactive oxygen and nitrogen species by macrophages and neutrophils that infiltrate inflammation sites. While pathologically, high levels of these reactive species cause damage to biological molecules, including DNA, nitric oxide at lower levels plays important physiological roles in cell signaling and apoptosis. This raises the question of inflammation-induced imbalances in physiological and pathological pathways mediated by chemical mediators of inflammation. On the other hand, CMG has been shown to exert antitumor growth activity through inhibition of cancer cell proliferation, angiogenesis, and inflammatory response. Therefore, studies have been performed to address the mechanisms of action of CMG at the genome-wide gene expression level (33). In one such study, Lewis lung carcinoma cells were treated with MGO or DMSO, and RNA was collected at five distinct time points. Microarray expression profiling was performed followed by computational analysis. For a number of selected genes, RT-PCR validation was performed in A549, HCT116, K562 human cancer cell lines of different tissue origin. Phosphatase and tensin homolog deleted on chromosome 1 (PTEN)-specific inhibition by a *bis*perovanadium compound was applied to validate its contribution to mastic oil-mediated antitumor growth effects. Exposure of Lewis lung carcinomas to MGO caused a time-dependent alteration in the expression of 925 genes. The expression profiles of heme oxygenase-1 (*HMOX1*), *PTEN*, and transcription factor-f7 (*E2F7*) genes were similarly altered by MGO in the majority of test cancer cell lines. Inhibition of PTEN partially reversed MGO effects on tumor cell growth, indicating a multitarget mechanism of action. Promoter analysis in a representative cluster revealed shared putative *cis*-elements suggesting a common regulatory transcription mechanism. These results provide novel evidence on the molecular basis of tumor growth inhibition mediated by MGO (33). In another study, cytotoxic activity of solid and liquid types of CMG against fibroblasts and 13 human tumor/leukemia cell types was assessed. The promyelocytic leukemia cell line HL-60 was the most sensitive to the cytotoxicity of CMG, followed by myeloblastic leukemia (ML-1, KG-1), erythroleukemia (K-562), oral squamous cell carcinoma (HSC-2, HSC-3, HSC-4), hepatocellular carcinoma (HepG2), glioblastoma (T98G, U87MG), and normal oral cells (gingival fibroblasts, pulp cells, periodontal ligament fibroblasts, mostly resistant). CMG was unable to induce differentiation of myelogenous leukemia cells into maturing cells, but induced apoptotic cell death, which was characterized by internucleosomal DNA fragmentation, caspase-3 activation, and a decline in the intracellular concentration of putrescine. The cytotoxicity of CMG towards leukemia cells did not diminish during its storage. On the other hand, CMG inhibited the spontaneous apoptosis of oral polymorphonuclear neutrophils (OPMN) (15).

Maspin is an epithelial-specific member of the serine protease inhibitor (serpin) superfamily and an endogenous inhibitor of HDAC1. Maspin can suppress tumor growth and metastasis *in vivo* and tumor cell motility and invasion *in vitro* (34-36). A study was conducted to determine whether CMG can regulate maspin expression in prostate cancer cells, and to further investigate the mechanisms involved in this regulatory system. RT-PCR and western blotting were used to detect maspin expression at the transcriptional and translational levels, respectively. The binding activity of negative androgen-responsive element (ARE) and positive (SP1) element in the maspin promoter were studied by electrophoretic mobility shift assay. CMG was found to enhance maspin promoter activity by suppressing the ARE binding activity, while enhancing SP1 binding activity, whereas the increased activity in the maspin promoter ultimately led to the up-regulation of both its mRNA and protein levels (37).

Accumulating evidence suggests that the androgen receptor (AR) may play an important role in the development and progression of prostate cancer. Therefore, there is an extensive effort to discover useful compounds that effectively attenuate the function of AR. On this regard, the effect of CMG on AR activity was investigated using an androgen-responsive prostate cancer cell line, LNCaP, as a model. The effect of CMG on the expression and function of the AR was determined by gene transfer, reverse transcriptase-polymerase chain reaction analysis, electrophoretic mobility shift assay, and western blot analysis. The results showed that CMG inhibited the expression of the AR at the transcriptional level, resulting in the down-regulation of both AR messenger RNA and protein levels. The function of the AR was inhibited, as reflected by the reduced expression of (NKX3.1) and prostate serum antigen (PSA), and by androgen-stimulated growth (38).

Another study investigated the effect of CMG on the proliferation of androgen-independent prostate cancer PC-3 cells, and further determined the mechanism(s) involved in this regulatory system, considering the NF-κB signal as the target. The expression of genes involved in the NF-κB signal pathway, including *cyclin D1,* inhibitors of κBs (*IκBα*), and phosphorylated AKT (*p-AKT*), were measured. In addition, transient transfection assays with the 5X NF-κB consensus sequence promoter were also used to test the effects of CMG. CMG reduced PC-3 cell growth by arresting the cells in the G_1 phase of the cell cycle and by suppressing NF-κB activity. The expression of cyclin D1, a crucial cell cycle regulator and an NF-κB downstream target gene, was reduced as well. Moreover, CMG decreased the p-AKT protein levels and increased the IκBα protein levels. In conclusion, CMG

inhibited the proliferation of PC-3 cells by blocking cell cycle progression *via* suppression of the NF-κB activity and therefore the NF-κB signaling pathway (39).

The antiproliferative and apoptotic effects of the anticancer drug gemcitabine combined with CMG were investigated in human pancreatic cancer cell lines. Cell proliferation and apoptosis were monitored using the methyl thiazolyl tetrazolium (MTT) assay and propidium iodine staining, respectively. The expression of BCL-2, BAX, NF-κB p65 subunit, and IκBα protein was measured using western blotting. The IκBα levels were increased, whereas NF-κB activation was blocked; the expression of BAX protein was substantially increased, but that of BCL-2 protein was down-regulated. It was concluded that the combination of gemcitabine-CMG may be an effective therapeutic strategy for pancreatic cancer (40).

Several natural dietary compounds can delay, prevent, or reverse the development of adenomas as well as the progression from adenoma to carcinoma and these events have been associated with modulation of signaling cascades, gene expression involved in the regulation of cell proliferation, and apoptosis and the suppression of chronic inflammation, metastasis, and angiogenesis (41). On this regard, we have demonstrated that CMG contains constituents that inhibit proliferation and induce death of HCT116 human colon cancer cells *in vitro*. CMG treatment induced cell arrest in the G_1 phase of the cell cycle, activation of pro-caspases 8, 9 and 3, and these events were p53- and p21-independent. Apoptosis induction by CMG was not inhibited in HCT116 cell clones expressing high levels of the anti-apoptotic protein, BCL-2, or dominant-negative FADD, thereby indicating that CMG induced cell death *via* an as yet-to-be identified pathway, unrelated to the death receptor- and mitochondrion-dependent pathways. The findings presented in the specific study suggest that CMG might be developed into a chemotherapeutic agent for the treatment of human colon and other types of cancer (42). Subsequently, we demonstrated that a hexane extract of CMG, He-CMG, possesses the ability to kill HCT116 cells *via* apoptosis with appearance of morphological features both typical and non-typical of apoptosis. Taken together, all results demonstrated that one or more constituents of the He-CMG extract had the ability to induce apoptosis in HCT116 cells *in vitro* (43) and further *in vivo* studies were warranted. Therefore, we extended the studies to investigate the *in vivo* anticancer activity of He-CMG against HCT116 tumors established in mice using the human colon cancer/immunodeficient SCID mouse model. He-CMG was administered at different schedules and doses ranging from 100 to 220 mg/kg body weight, and the tumor growth (size) was monitored. He-CMG administered daily at a dose of 200 mg/kg, for four consecutive days (followed by three days without treatment) inhibited tumor growth by approximately 35% in the absence of toxicity (side effects) after 35 days. We concluded that He-CMG possesses an antitumor activity against human colorectal cancer under the experimental conditions of the study, and that the extent of suppression and toxicity by a specific He-CMG dose depends on the schedule of administration (44). However, this study does not identify constituent(s) of the He-CMG associated with the HCT116 tumor growth inhibition.

Other Properties of CMG

Toxicity in rats. Dietary toxicity of CMG was studied in male and female F344 rats, fed with various CMG doses. Body weights were significantly reduced in the high-dose-treated group. Altered serum biochemistry parameters included increases of total proteins, albumin, and total cholesterol in both sexes, and of γ-GTP in females only. However, macroscopic examination at necropsy revealed no gross lesions, and microscopic examination also revealed no treatment-related findings in any organs examined. It was concluded that the administration of CMG has an adverse effect level of 0.67% in the diet (45).

Treatment of patients with dyspepsia. Dyspepsia is a common term used for a heterogeneous group of abdominal symptoms (46). Functional dyspepsia (FD) is a very common condition with a high prevalence throughout the world, adversely affecting the quality of life of patients (47). Multiple mechanisms such as abnormal gastric emptying, visceral hypersensitivity, impaired gastric accommodation, and central nervous system factors are likely involved. Currently, the possibilities of pharmacological therapy for FD are still limited. Herbal remedies are increasingly popular for the treatment of FD. The efficacy of CMG in patients with FD has been investigated. In one study, patients fulfilling criteria for FD were randomly assigned to receive either CMG or placebo. FD was assessed using the Hong Kong index of dyspepsia. The symptom score after treatment was significantly lower in the CMG group than in the placebo group. There was a marked improvement of symptoms in 40% of patients receiving placebo and in 77% of patients receiving CMG. It was concluded that CMG significantly improves symptoms in patients with FD compared to placebo (47).

CMG application in surgery. In this section, we discuss the use of CMG in surgical strips and tapes. Surgical adhesive strips are often used for closure of some wounds or to minimize tension on sutures after closure. To increase the adhesive power of benzoin, a compound tincture of benzoin (CTB) was compared with a preparation containing CMG (Mastisol). The study clearly demonstrated that the latter preparation provided markedly more adhesive strength than that obtained with CTB alone (48).

In another study, strip reinforcement with/without CMG did not provide any additional strength when sutures were used.

CMG increased the adherence of strips, which was important when strips were the only means of wound closure (49). This observation was confirmed in another study, which demonstrated that the combination of CMG and 0.5-inch Steril-Strips provides the strongest adhesion (50), and that this type of application should also prove useful when other types of surgical dressings must be anchored in place.

Future Perspectives

Although very little is known about the molecular mechanisms associated with the various activities of CMG it is likely that CMG/MGO directly or indirectly modulates signaling cascades, gene expression involved in the regulation of cell proliferation, differentiation, apoptosis and the suppression of chronic inflammation, metastasis, and angiogenesis. Furthermore, the ability of CMG to directly or indirectly target a large number of various components or events of specific molecular mechanisms may ultimately result in chemoprevention or cure of a disease, as discussed above. On this regard, CMG may be able to regulate inflammatory signals that are required for promoting or suppressing the activation of key molecules or events of a particular mechanism. The site of intervention of CMG on the regulation of the inflammatory signals may be an early or late event. An example of early hypothetical intervention in a cytokine-regulated mechanism would be the induction of inflammatory signals that activate the cAMP-response element-binding protein, CREB, a transcription factor that subsequently mediates the transcription of several immune-related genes containing a cAMP-responsive element, including the cytokines IL-2, IL-6, and IL-10, involved in proliferation, survival, and differentiation (51, 52). The importance of early-event implication of pro-inflammatory elements has been shown in studies of gene-deficient mice and cells in which activation of pro-inflammatory proteins (inflammasomes) is a prerequisite in the maturation and secretion of pro-IL-1β and pro-IL-18 among other substrates in a host of responses to a wide range of microbial pathogens, inflammatory diseases, cancer, and metabolic, and autoimmune disorders (53). In contrast, inflammasomes may provoke significant release of IL-1β and IL-18 by resting macrophages infected with *Francisella tularensis*, a facultative intracellular pathogen and potential biothreat agent, which results in the extraordinary virulence of this organism (54). However, no studies have been published regarding the interaction of CMG with inflammasomes. Furthermore, an example of CMG-mediated late intervention has already been mentioned in this article. CMG inhibited the expression of iNOS and COX-2 at both the mRNA and protein levels (20). In general, CMG may be able to modulate the activity of various cytokines, including IL-10 family cytokines, which are essential for promotion of innate immune responses from tissue epithelia, to maintain the integrity and homeostasis of tissue epithelial layers, limit the damage caused by viral and bacterial infections, and also facilitate the tissue-healing process in injuries caused by infection or inflammation (51).

Finally, in preliminary studies we have shown that CMG induces apoptosis of human colorectal cells *in vitro* (42, 43) and attenuate growth of human colon tumors, established as xenografts in immunodeficient mice (44). It remains to be seen whether pre-treatment of immunodeficient mice with CMG will completely block the establishment of the human colon tumor xenografts or may allow for establishment, but evoke delay of tumor growth to an extent that is not terminal for the animal. In other words, it would be important to determine the potential of CMG to serve as a chemopreventive agent and inhibit growth of human colon cancer as well as cancer derived from diverse tissues. CMG/GMO chemoprevention and chemotherapy of gastrointestinal cancers as well as liver and heart diseases present many exciting possibilities. Extraction of active ingredient(s) and pharmaceutical formulation procedures need to be evaluated, investigated, and developed. Nevertheless, the prospects for CMG as a therapeutic agent are indeed promising.

It has been demonstrated that the majority of the resins obtained from *P. lentiscus* consists of the phytochemicals, α-pinene, β-pinene, β-myrcene, limonene, *trans*-caryophyllene and camphene. Therefore, it is reasonable to investigate whether these constituents possess the various abilities described above. One possibility is that a simple constituent alone exhibits one or more of the various abilities. It is also possible that the observed medical/pharmaceutical properties of CMG are not associated with one but rather two or more of these phytochemicals. It is also very likely that various combinations of CMG constituents possess activities against specific diseases. Therefore, it would be of interest to initially determine whether each one of the major constituents, alone and in synergy with another major or minor constituent, have the ability to reproduce or enhance the activities reported for CMG and MGO. It should be noted that the concentrations of the constituents in the mixtures should be the same as in the whole CMG or MGO. In this manner, we have demonstrated that the CMG monoterpene, camphene, reduces total plasma cholesterol, LDL and triglycerides in rats *via* a mechanism independent of HMG-CoA reductase activity (30). Finally, it is also highly possible that a specific CMG activity is associated with a minor constituent. This possibility will make the task difficult or impossible to carry out, since large quantities of this minor constituent will be difficult to prepare in adequate quantities for studies. However, many of these constituents are commercially available, and various combinations of the known constituents can be prepared at the concentrations identical to those present in CMG or MGO. For example, one approach to identify the possible active CMG constituent(s) against human cancer would be the method of 'sequential fractionation', and testing each fraction for biological activity.

The positive fractions would then be analyzed by high-performance liquid chromatography mass spectroscopy to identify common constituent(s). The positive fraction with the least number of constituents could be tested both *in vitro* and *in vivo*.

Another difficult task will be to determine the proper solvent as a carrier in the studies, since most of the known major constituents are terpenes, which are phytochemicals insoluble in aqueous solutions. Any carrier used should be on the list of carriers recommended by the Experimental Therapeutics Program of the NCI, NIH, USA. In a preliminary study of treatment of human colon cancer xenografts we have successfully used such a carrier (44).

In general, the published reports on the numerous and diverse medical and pharmaceutical properties of CMG warrant its further research.

Authorship Contributions

K.S. Dimas, P. Pantazis, and R. Ramanujam wrote or contributed equally to the writing of the manuscript.

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