Dietary Chlorophyllin Is a Potent Inhibitor of Aflatoxin B₁ Hepatocarcinogenesis in Rainbow Trout¹

Vibeke Breinholt, Jerry Hendricks, Cliff Pereira, Daniel Arbogast, and George Bailey²

Departments of Food Science and Technology [V. B., J. H., D. A., G. B.] and Statistics [C. P. J., Oregon State University, Corvallis, Oregon 97331

ABSTRACT

Epidemiological and experimental evidence indicates a strong relationship between diet and cancer. The purpose of this study was to examine the potential of chlorophyllin (CHL), a food-grade derivative of the ubiquitous green plant pigment chlorophyll, to inhibit experimental carcinogenesis. We report that CHL is a potent, dose-responsive inhibitor of aflatoxin B₁ DNA adduction and hepatocarcinogenesis in the rainbow trout model when fed with carcinogen. CHL neither promoted nor suppressed carcinogenesis with chronic postinitiation feeding. By molecular dosimetry analysis, reduced aflatoxin B₁-DNA adduction accounted quantitatively for reduced tumor response up to 2000 ppm dietary CHL, but an additional protective mechanism was operative at 4000 ppm CHL. The finding of potent inhibition (up to 77%) at CHL levels well within the chlorophyll content of some green leafy vegetables may have important implications in intervention and dietary management of human cancer risks.

INTRODUCTION

Diet has been shown consistently to be an important determinant of human cancer risk (1–4). Although much attention has been given to dietary carcinogens and promoters, recent evidence indicates that protective factors within the diet are also important mediators of cancer risk. Numerous chemical constituents in fruits and vegetables have now been purified and shown to protect against carcinogenesis in experimental animal models (5–8), and several candidate chemopreventives are now in clinical trials. The potential anticarcinogenic activity of chlorophyll, the ubiquitous pigment in green plants, is of considerable interest because of its relative abundance in green vegetables widely consumed by humans. Chlorophyll and its food grade derivative CHL² have been shown to exert profound antimutagenic behavior against a wide range of potential human carcinogens (9–15). CHL has been used historically in the treatment of several human conditions, with no evidence of human toxicity (16–18).

Several CHL antimutagenesis mechanisms have been postulated, including tight complex formation with parent mutagens or their activated intermediates, scavenging of radicals and active oxygen species, and suppression or interference with metabolic activation by specific cytochrome(s) (P-450) and other drug-metabolizing enzymes (19–25). CHL was shown in vivo to efficiently inhibit preneoplastic target organ DNA adduction by AFB₁ in trout (22) and 2-amino-3-methylimidazo[4,5-f]quinoline in the rat (26). We are not aware, however, of any published studies demonstrating inhibition of tumor response by chlorophyll or its derivatives. The purpose of this study was to examine the potential of CHL to inhibit hepatocarcinogenesis induced by AFB₁, a potent mycotoxin associated with human liver cancer (27). The rainbow trout, a model that we have developed to quantify the relationship between carcinogen dose, anticarcinogen dose, level of target organ DNA adduction, and final tumor response (28), was used in the current study.

MATERIALS AND METHODS

Animals and Tumorogenesis Protocols. Fingerling rainbow trout (9490) of 1.5–2.0 g body weight were randomly distributed into 73 tanks of 130 fish. The fish were acclimatized for 2 weeks receiving 1 daily feeding of a semipurified control diet (OTD) (29). During the following 2 weeks the fish were fed varying doses of AFB₁ ranging from 10 to 160 ppb in combination with 0, 500, 2000, or 4000 ppm CHL (see Table 2). An additional 3 tanks received OTD diet only, and 2 tanks per inhibitor dose served as CHL controls and were fed the appropriate CHL dose only. To examine CHL postinitiation effects, another 4 tanks received 20 ppb AFB₁ for 2 weeks, followed by 7 days on OTD diet. For the remaining 9 months duplicate groups of animals from this treatment were exposed to either 2000 or 4000 ppm CHL incorporated into OTD. In a second study, duplicate groups were initiated by 4 weeks of 80 ppb dietary AFB₁, and then fed OTD, 1500 ppm CHL, 1500 ppm indole-3-carbinol as a positive control for promotion, or 1500 ppm CHL plus 1500 ppm indole-3-carbomin combined, for 30 weeks. All diets were weighed out in individual daily rations and kept frozen at −20°C. Prior to feeding the diets were thawed at 5°C in the dark. The diet ration, fed once a day, was based on 2% (dry weight diet) of the body weight and was totally consumed without wastage. During preparation of the diets and feeding, exposure to light was minimized to reduce light degradation of AFB₁ and CHL. All trout were housed and treated according to guidelines from NIH and protocols approved by the Animal Welfare Committee at Oregon State University.

Determination of Hepatic AFB₁-DNA Adduction in Vivo. All the toxic diets contained 5 ppb radioactive AFB₁ (18.84 mC/mol) and varying amounts (5–155 ppb) of unlabeled AFB₁. The diets were prepared by dissolving CHL in 10 ml of water, followed by the addition of AFB₁ contained in 50 µl of 95% ethanol. The two compounds were allowed to interact for approximately 5 min before addition of salmon oil, water, and OTD dry ingredients. The final level of radioactive AFB₁ in the diet was determined by digesting 0.2–0.5-g diet samples in 15 ml NCS (Amersham) tissue solubilizer at 50°C, and counting 50–125 aliquots in organic scintillation fluid. After 14 days exposure to the various carcinogen and inhibitor combinations, 15 fish were randomly selected from each tank. The fish were killed by decapitation, and the livers were removed, pooled in three groups of five, and placed on dry ice prior to storage at −80°C. During the 2 weeks of AFB₁ exposure, the fish gained an average 0.53 g, and the weight gain was not different among the various treatment groups as determined by ANOVA (P > 0.50). The liver somatic index was likewise not affected by any of the exposure combinations relative to the OTD control groups (P > 0.40). Liver DNA was purified, and specific activity for AFB₁-DNA adduction was determined as described elsewhere (28). We have been unable to detect any CHL-mediated changes in trout hepatic AFB₁-metabolizing enzymes that might result in different DNA adducts such as CYP1A, which catalyzes AFM₁ formation. However, our previous studies (e.g., Ref. 28) with other anticarcinogens have failed to reveal any effect of blocking agents on the profile of trout hepatic AFB₁-DNA adducts, which are almost entirely the 8,9-dihydro-8-(N²-guanany)-9-hydroxy-aflatoxin B₁ adduct and its ring-opened derivatives. Therefore, we determined only total AFB₁-DNA binding rather than quantifying individual adducts in this study.

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² To whom requests for reprints should be addressed, at Department of Food Science, Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602.

³ The abbreviations used are: CHL, chlorophyllin; AFB₁, aflatoxin B₁; OTD, Oregon Test Diet; CI, 95% confidence interval; T₉₀, 90% tumor response; DBI, DNA-binding index.
**RESULTS**

**Effects of CHL on AFB, Hepatocarcinogenesis.** The percentages of each tumor type were assessed to determine whether CHL treatment caused any shift in tumor phenotype. The distribution of tumor types seen in the 160 ppb AFB, group (Table 1) was found to be similar to that reported previously for AFB, (32), 7,12-dimethylbenz(a)anthracene, benzo(a)pyrene, or N-nitrosodimethylamine (33-35). Since we have never observed an effect of AFB, dose on tumor phenotype distribution, only the highest AFB, dose was examined histologically in this study. The lower number of tumors observed in the 160 ppb AFB, plus 4000 ppm CHL group tended to skew the distribution of tumors into fewer groups giving higher percentages but, within this limitation, the types of tumors produced appears similar to that with AFB, alone. Dietary CHL up to 4000 ppm for 9 months induced no obvious toxicity such as increased mortality rate, altered hepatosomatic index, or impaired growth rate that might contribute to the observed tumor responses (data not shown). Although complete histology was not conducted for other groups, there is no reason to believe that CHL at lower doses would have changed the distribution of tumor phenotypes, only the number of tumors.

Tumor incidences among treatment groups given AFB, alone, or in combination with CHL given before and during AFB, exposure, are shown in Table 2. For completeness all 19 treatment groups in the original design are shown. The data can be analyzed in several ways to examine CHL efficacy. Simple pairwise comparisons among groups receiving higher AFB, doses (e.g., 160 ppb AFB, versus 160 ppb AFB, plus 4000 ppm CHL) show clearly that CHL effectively inhibited AFB, carcinogenesis in this study. For example, tumor responses in groups cofed 4000 ppm CHL and 40, 80, or 160 ppb AFB, were only 23–33% (P ≤ 0.01 each comparison) of the responses of groups fed AFB, alone. Tumor response in the controls receiving 10 or 20 ppb AFB, alone were too low to reveal any CHL protection.

Modeling the dose-response relationships can provide useful quantitative summaries of the effects of CHL over the range of carcinogen and inhibitor doses studied. Tumor incidences were modeled here for 40, 80, and 160 ppb AFB, where there was evidence of CHL effects. Our experiment, which used doubling carcinogen doses and triplicate tanks of 100 animals each for each AFB,/CHL treatment, was designed to estimate true incidences of 5% or greater. However, the overall tumor response in the present study was somewhat lower than that seen previously with AFB, doses in this range (28). As a consequence, AFB, doses ≤20 ppb were not included in the modeling because of the low incidences (2% or less for all treatments) and lack of evidence for any CHL effects at these doses.

Two types of models were fitted to the data with similar results. Incidence was first modeled as directly proportional to AFB, dose (P = kx) with a potentially different slope (k) for each CHL concentration studied. Tumor incidences were modeled here for 40, 80, and 160 ppb AFB, where there was evidence of CHL effects. Our experiment, which used doubling carcinogen doses and triplicate tanks of 100 animals each for each AFB,/CHL treatment, was designed to estimate true incidences of 5% or greater. However, the overall tumor response in the present study was somewhat lower than that seen previously with AFB, doses in this range (28). As a consequence, AFB, doses ≤20 ppb were not included in the modeling because of the low incidences (2% or less for all treatments) and lack of evidence for any CHL effects at these doses.

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Models for tumor incidence data were fit by maximum likelihood and models were compared using likelihood ratio tests (31) in the SAS Genmod procedure. DNA-binding data were logarithmically transformed and analyzed using linear models and ANOVA in the SAS GLM procedure.
of 500, 2000, and 4000 ppm, respectively.

obtained from the AFB1-CHL co-fed groups of fish were successfully scale. The curves (Fig. 1, inset) describing the tumor incidences (28). To do this, data were analyzed on the logit incidence-log dose potency examines modulator effects on logit-derived TD50 values (CI = 19–47%), and 77% inhibition (CI = 68–84%) for CHL doses tested decreased the incidence of tumor-bearing animals along did not approach saturation in this experiment. CHL at the various doses tested decreased the incidence of tumor-bearing animals along the AFB1 dose-response curve, giving an overall protection against hepatocarcinogenesis that is reflected in decreased slopes relative to the AFB1 only control curve (Fig. 1). In this model, percentage inhibition of tumor response at any CHL dose (x) can be defined as 100 [1 – slope_CHL/slope_control]. Using this analysis, percentage inhibition in tumor incidence was determined to be 35 (CI = 13–53%), 34 (CI = 19–47%), and 77% inhibition (CI = 68–84%) for CHL doses of 500, 2000, and 4000 ppm, respectively.

An alternative we have previously used to quantify modulator potency examines modulator effects on logit-derived TD50 values (28). To do this, data were analyzed on the logit incidence-log dose scale. The curves (Fig. 1, inset) describing the tumor incidences obtained from the AFB1-CHL co-fed groups of fish were successfully modeled as straight-line, parallel-offset relationships. Each CHL dose displaced the response curve horizontally toward higher AFB1-doses, as previously seen for indole-3-carbinol anticarcinogenesis (28). That the response lines could be successfully modeled as parallel provides evidence that the efficacy of CHL anticarcinogenesis was independent of carcinogen dose, over the range of tumor incidences (7%-50%) examined here. The AFB1 doses required for TD50 values at each level of CHL were obtained by extrapolation of each data set to 50% incidence. (Actually, for the parallel logistic regression model, extrapolation to 50% is not essential, since the relative potency will be the same for any choice of percentile p in comparing TD50 values). The TD50 values for the 0, 500, 2000, and 4000 ppm CHL groups so obtained were 298 (CI = 232–381), 442 (CI = 284–688), 449 (CI = 326–619), and 1135 (CI = 678–1900) ppb AFB1, respectively.

Percentage inhibition of tumorigenesis can then be summarized as [100 (1 – TD50control/TD50chl)] at each dose of CHL tested. The CHL dose-response potency for inhibition of tumorigenesis estimated by this method was 33 (CI = 6–52%), 34 (CI = 17–47%), and 74% (CI = 62–82%) at 500, 2000, and 4000 ppm CHL respectively, similar to the values obtained from the approach where incidence was modeled as directly proportional to dose. The models are closely related, and it is not surprising that they would give similar results for.

Fig. 1. Tumor incidence versus aflatoxin dose, with varying levels of CHL cotreatment. Symbols represent 0 (A), 500 (C), 2000 (D), or 4000 (L) ppm dietary CHL treatments. Points, mean tumor incidences for 3 tanks of 100 fish each for each AFB1 dose and CHL pretreatment level (data from Table 2); bars, SE. Tumor incidences were modeled for the AFB1 doses ≥40 ppb, where there was evidence for CHL effects. Lines, maximum likelihood fits for modeling incidence as directly proportional to dose (P = mx) with a different slope (m) for each CHL pretreatment. The model explains the variation in incidence for the data points examined (lack of fit versus full model; 7 degrees of freedom; P = 0.28) and indicates highly significant differences among treatment groups (test of common slope; 3 degrees of freedom; P < 0.0001), although there is no evidence for differences between the 500 and 2000 ppm CHL groups. Inset, pooled tumor incidence versus aflatoxin dose with scales changed to logit and log, respectively. Inset lines, maximum likelihood fits for a parallel linear logistic regression model. This model also explains the variation in tumor incidence (lack of fit versus full model, 6 degrees of freedom, P = 0.26; test of homogeneity of slopes, 3 degrees of freedom, P = 0.15) and indicates highly significant differences among treatment groups (test of common intercepts, 3 degrees of freedom, P < 0.0001), although there is no evidence for difference between the 500 and 2000 ppm CHL groups.

Fig. 2. Inhibition of AFB1-DNA adduction by CHL. Fish were exposed for 2 weeks to AFB1 doses ranging from 10 to 160 ppb in combination with 0 (A), 500 (C), 2000 (D), or 4000 (L) ppm dietary CHL treatments. Symbols represent 0 (A), 500 (C), 2000 (D), or 4000 (L) ppm CHL treatments. Points, geometric mean of 6-9 individual determinations (backtransformed from the log scale) for each AFB1 dose and CHL pretreatment level; bars, multiplicative SE. Responses were modeled for AFB1 doses of 40–160 ppb, where there was evidence of CHL effects. [By ANOVA of the log transformed data, the effect of CHL depended on dose of AFB1 (test for interaction, P < 0.005), with strong evidence for CHL effects at 40, 80, and 160 ppb AFB1 (P < 0.001 for each) and no evidence of CHL effects at 10 or 20 ppb AFB1 (P > 0.2 for each)]. Lines are from log scale analysis [log(y/x) = log(k)x] equivalent to modeling DNA binding (y) as directly proportional to dose (x) of AFB1 (y = kx) with a different slope (k) for each CHL level. The model adequately explains the variation in DNA binding for the data examined (lack of fit versus saturated model, 7 degrees of freedom, P = 0.22) and indicates highly significant differences among control and CHL treatment groups (3 degrees of freedom, P < 0.0001).
ppm fits the data well (lack of fit versus full model, 8 degrees of freedom, P = 0.54), and ppm CHL pretreatments, P > 0.4 using a normal approximation with assumption of from each other only for the 4000 ppm CHL pretreatment (P < 0.0001; for 500 and 2000 scale) for each AFB, dose and CHL pretreatment. Both analyses indicate that inhibition of DNA adduction can account for the observed inhibition in tumor incidence for all CHL fractions of tumorigenesis, for three doses of CHL. Points, estimates and 95% confidence intervals from DNA adduction modeling described in Fig. 2, and from logistic regression modeling of tumor response described in Fig. 1. The symbols are the same as in Figs. 1 and 2. Inset, pooled tumor incidence (logit scale) versus mean DNA binding (log scale) for each AFB, dose and CHL pretreatment. Both analyses indicate that inhibition of DNA adduction can account for the observed inhibition in tumor incidence for all CHL pretreatments except 4000 ppm. The DBI ratio and TDS0 ratio were significantly different from each other only for the 4000 ppm CHL pretreatment (P < 0.0001); for 500 and 2000 ppm CHL pretreatments, P > 0.4 using a normal approximation with assumption of approximate independence of DBI and TDS0 estimators. For the logistic regression analysis a parallel line model with a single line for all CHL treatments except the 4000 ppm line is significantly shifted away from the rest (P < 0.0001).

low incidences and a limited range of doses. An important distinction is that while the data can be successfully fitted to a parallel-offset model, they are not sufficiently precise to exclude other relationships; for example, if each CHL curve were modeled as an independent data set, the four logit incidence-log dose curves would not likely have identical slopes.

CHL Effects on Hepatic AFB1-DNA Adduction in Vivo. The covalent binding of dietary AFB1 to hepatic DNA in vivo was also examined in this study. As in previous experiments (e.g., Ref. 28), hepatic DNA adduction in the AFB1-only positive controls was directly proportional to AFB1 dietary concentration at the completion of a 14-day initiation exposure (Fig. 2). Exposure of animals to varying doses of CHL beginning 2 weeks prior to AFB1 treatment produced a series of curves of decreasing gradient with increasing CHL dose. On the basis of the higher AFB1 doses (40, 80, and 160 ppb), the slope of each of the curves defined estimated DBIs of 0.176 (CI = 0.162–0.192), 0.136 (CI = 0.123–0.151), 0.123 (CI = 0.113–0.134), and 0.111 (CI = 0.102–0.122) μmoles adducts/mol DNA/ppb AFB1 for 0, 500, 2000, and 4000 ppm CHL, respectively. The potency of each CHL dose to inhibit AFB1-DNA adduction can be defined in percentage terms as 100 (1 – DBI_{CHL}/DBI_{control}). Based on this analysis, AFB1-DNA adduction in liver was inhibited 23 (CI = 12–32%), 30 (CI = 21–38%), and 37% (CI = 29–44%) at dietary CHL levels of 500, 2000, and 4000 ppm, respectively.

Molecular Dosimetry. We were interested to determine mechanistically if CHL-mediated reduction in AFB1-DNA adduction could account totally for CHL inhibition in tumor response. By molecular dosimetry analysis (logit incidence versus log DNA adducts), which involves the fewest model assumptions, tumor response among all treatment groups appeared to be reasonably well predicted by AFB1-DNA adduct levels in all treatment groups, with the apparent exception of two data sets at 4000 ppm CHL (Fig. 3, inset). This analysis suggests that blocking of DNA adduction is the sole or predominant CHL anticarcinogenic mechanism at all but the highest CHL dose. The relationship between the CHL-induced changes in AFB1-DNA adduction during initiation and final reduction in tumor outcome at 9 months is effectively visualized by plotting the logit-derived TDS0 ratio (TDS0_{control}/TDS0_{CHL}) versus the DBI ratio (DBI_{CHL}/DBI_{control}) at each dose of CHL. In principal, the estimates will fall on a line of slope unity if CHL inhibition accounted quantitatively for inhibition of tumorigenesis at all doses of CHL tested. As seen in Fig. 3, estimates defining the relationship between TDS0 ratios and DBI ratios at the two lower doses of CHL were close to the theoretical line of unity slope, but the estimate at 4000 ppm CHL was significantly (P < 0.001) below the line. This indicates that at lower CHL doses inhibition of AFB1-DNA adduction accounts largely or entirely for the anticarcinogenic effect, whereas at higher doses reduced carcinogen-DNA adduction accounts for only about 50% of CHL tumor reduction. We interpret these results to indicate the presence of at least two CHL inhibitory mechanisms, one of which gains in relative contribution with increasing CHL dose. The utility of this quantitative anticarcinogenic approach lies in its ability to detect the operation of mechanisms, perhaps unexpected, in addition to or other than simple blocking of carcinogen-DNA damage, and to gain some numerical estimate of their relative importance.

Postinitiation Effects of CHL. We also investigated the postinitiation behavior of CHL in order to assess the effects of long-term dietary intake of CHL. The results of two separate studies are shown in Table 3. Experiment 1 involved a low level of initiation (1% incidence in the initiation only control), which permitted testing for promotional activity near a subcarcinogenic initiator level. No significant postinitiation promotional activity of CHL was detected in this study when CHL was fed for 9 months at 2000 or 4000 ppm. Because of the low level of initiation in the AFB1 control, this experiment could not detect any possible activity of CHL as a postinitiation

![Graph](https://via.placeholder.com/150)

Fig. 3. Relationship between CHL fractional inhibition of AFB1-DNA adduction and fractional inhibition of tumorigenesis, for three doses of CHL. Points, estimates and 95% confidence intervals from DNA adduction modeling described in Fig. 2, and from logistic regression modeling of tumor response described in Fig. 1. The symbols are the same as in Figs. 1 and 2. Inset, pooled tumor incidence (logit scale) versus mean DNA binding (log scale) for each AFB, dose and CHL pretreatment. Both analyses indicate that inhibition of DNA adduction can account for the observed inhibition in tumor incidence for all CHL pretreatments except 4000 ppm. The DBI ratio and TDS0 ratio were significantly different from each other only for the 4000 ppm CHL pretreatment (P < 0.0001); for 500 and 2000 ppm CHL pretreatments, P > 0.4 using a normal approximation with assumption of approximate independence of DBI and TDS0 estimators. For the logistic regression analysis a parallel line model with a single line for all CHL treatments except the 4000 ppm line is significantly shifted away from the rest (P < 0.0001).
suppressing agent, nor did it test the possibility that CHL-enhancing activity could be operative only at higher levels of initiation. In addition, experiment 1 did not include a positive control for promotion. Experiment 2 addressed these issues by using a higher level of initiation (29% incidence in the AFB1 only control), and by including the ambivalent modulator indole-3-carbinol, a well known postinitiation promoter in this model (36). Dietary CHL fed for 8 months at 1500 ppm clearly did not promote (Table 3) and, if anything, gave some indication of suppressing activity. As a further test of suppression, CHL was included with I3C in a coexposure protocol. At the CHL dose tested, there was no significant suppression of I3C promotion.

DISCUSSION

Mechanisms of CHL Anticarcinogenesis. One potential mechanism for CHL blocking of AFB1 initiation may lie in the ability of CHL to associate in vitro with carcinogens having somewhat planar, conjugated characteristics. It has been suggested that the formation of such complexes in the diet or in the digestive tract may be responsible for reduced carcinogen uptake (26) and consequent reduced total body burden in CHL-fed animals. In support of this, analysis of CHL-ABF1 interaction in vitro by fluorescence-quenching measurements indeed indicates a relatively strong complex (Kd of 1.4 μM) with a formation rate that, from stop-flow cytometry measurements, is diffusion controlled and essentially independent of pH or temperature. Moreover, pharmacokinetic experiments using aflatoxin B1, an analogue of AFB1, that binds poorly to macromolecules, has provided direct evidence in vivo for CHL-mediated reduction in AFB1 target organ access. This mechanism, which may account for the inhibitory component resulting in reduced AFB1-DNA adduction, should be expected to apply as well to humans as to the trout model. The additional CHL activities necessary to account for CHL anticarcinogenic behavior at higher doses in the present experiments are not understood but might include mitosuppression or killing of potential target cells for AFB1. At present there is no direct evidence for the participation of blocking mechanisms operative in vivo within target cells as a result of CHL uptake and distribution into liver or other target organs, especially since the biodistribution and metabolism of CHL in vivo in carcinogenesis protocols have yet to be studied. However, a recent preliminary report of CHL chemoprotection in benzo(a)pyrene-initiated mouse skin tumorigenesis suggests sufficient systemic CHL distribution for such mechanisms to be operative in the whole animal (37).

CHL Potency and Relevance to Human Dietary Chlorophylls. These experiments were conducted using a typical commercial CHL preparation, which was determined to be 34% CHL and 66% neutral salts. CHL was selected for these initial studies rather than a pure chlorophyll a or b preparation because the latter are exceedingly unstable and cost prohibitive for such large-scale tumor experiments, especially since the biodistribution and metabolism of CHL in vivo in carcinogenesis protocols have yet to be studied. However, a recent preliminary report of CHL chemoprotection in benzo(a)pyrene-initiated mouse skin tumorigenesis suggests sufficient systemic CHL distribution for such mechanisms to be operative in the whole animal (37).

appropriate than chlorophyll itself to mimic in vitro the interaction of mutagens and chlorophyll in the human gastrointestinal tract in vivo. When corrected for actual chlorin content, the dose of CHL chlorins required to give an overall protection against AFB1 carcinogenesis of 70–80% in these studies was less than 1500 ppm. By comparison, the reported concentration of chlorophylls in spinach isolates is in the range of 0.15–6.0% (1,500–6,000 ppm), depending on agronomic conditions (43, 44). Hence, the CHL treatments used in this study are well within the range of chlorophyll found in human foods. The results thus suggest a potential role for this common dietary phytochemical in protection against certain classes of genotoxic dietary carcinogen, although it is not clear that effective intake could be achieved in a balanced diet without supplementation.

The absence of CHL promotion in the present study is contrary to a recent report of CHL-promotional activity in the rat colon carcinogenesis model (45). The reason for the discrepancy between the two models is not known. In a recent study, CHL given chronically in drinking water inhibited rather than enhanced the incidence of rat hepatic and colonic tumors initiated by extended treatment with 2-amino-3-methylimidazo[4,5-f]quinoxaline. However, this was a coexposure and not a pure postinitiation protocol. It will be important that the reported promotional behavior of CHL be verified and that the associated mechanisms be defined in order to establish the possible human relevance of this observation. For example, one testable hypothesis is that colonic bacterial degradation of CHL may provide release of sufficient free Cu2+ to support prooxidative promotional events in at least some colonic tumor models. Natural dietary chlorophylls do not to our knowledge contain significant copper, and we are not aware of any reports of tumor promotional activity of these commonly consumed phytochemicals.

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CHLOROPHYLLIN DOSE-RESPONSIVE CHEMOPREVENTION


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