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The coffee-specific diterpenes cafestol and kahweol protect against aflatoxin B1-induced genotoxicity through a dual mechanism

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The diterpenes cafestol and kahweol (C&K) have been identified in animal models as two potentially chemoprotective agents present in green and roasted coffee beans. It has been postulated that these compounds may act as blocking agents by producing a co-ordinated modulation of multiple enzymes involved in carcinogen detoxification. In this study, we investigated the effects of C&K against the covalent binding of aflatoxin B1 (AFB_1) metabolites to DNA. Male Sprague–Dawley rats were treated with increasing amounts of a mixture of C&K in the diet (0–6200 p.p.m.) for 28 and 90 days. A dose-dependent inhibition of AFB_1 DNA-binding was observed using S9 and microsomal subcellular fractions from C&K-treated rat liver in an *in vitro* binding assay. Significant inhibition was detected at 2300 p.p.m. and maximal reduction of DNA adduct formation to nearly 50% of the control value was achieved with 6200 p.p.m. of dietary C&K. Two complementary mechanisms may account for the chemopreventive action of cafestol and kahweol against aflatoxin B1 in rats. A decrease in the expression of the rat activating cytochrome P450s (CYP2C11 and CYP3A2) was observed, as well as a strong induction of the expression of the glutathione-*S*-transferase (GST) subunit GST Yc2, which is known to detoxify highly the most genotoxic metabolite of AFB_1. These data and the previously demonstrated effects of C&K against the development of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced carcinogenesis at various tissue sites suggest the potential widespread effect of these coffee components against chemical carcinogenesis.

Introduction

Aflatoxin B1 (AFB_1*) is a secondary metabolite produced by some strains of the fungi *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. It is a potent hepatotoxin and hepatocarcinogen in various mammalian species (1–5). Several epidemiological studies suggest its close association with the high incidence of liver cancer in exposed populations where contamination of foodstuffs with AFB_1 is common (6,7).

There are significant variations in the susceptibility of different animal species to AFB_1-mediated toxicity. Humans and rats are very sensitive to AFB_1 in contrast with mice which are relatively insensitive. Differences in aflatoxin biotransformation pathways are critical determinants underlying these variations in species sensitivities to AFB_1-induced carcinogenesis. AFB_1 is metabolized by the cytochrome P450 (CYP450) mixed-function mono-oxygenase system to a variety of reduced and oxidized derivatives. The predominant phase I metabolites formed in mammals are aflatoxin M1 (AFM_1), aflatoxin Q1 (AFQ_1), aflatoxin P1 (AFP_1) and aflatoxin B1-8,9-epoxide (AFBO) (2–5). The first three are polar products of AFB_1 that do not have the carcinogenic characteristics of AFBO, an unstable reactive metabolite which readily forms adducts with nucleophilic sites in DNA (8). The formation of these adducts can lead to mutations in proto-oncogenes and tumour suppressor genes and plays an important role in the initiation of hepatocellular carcinoma. However, AFBO can be inactivated prior to its reaction with DNA by conjugation with glutathione (GSH) mediated by glutathione-*S*-transferase (GST) isoenzymes (9–11).

There is increasing interest in the identification of chemoprotective agents that can reduce the genotoxicity of AFBO either by inhibiting its formation or by promoting its detoxification. Several studies in experimental animal models have provided support for the protective effects of coffee against certain chemical carcinogens and have led to the identification of some coffee components that may be responsible for these effects. The lipidic diterpenes cafestol and kahweol (C&K) have been identified as two potentially chemoprotective agents in green and roasted coffee beans (12,13). The mechanisms responsible for the chemoprotective effects of coffee or its diterpene constituents have not been completely elucidated. However, one hypothesis is that C&K act as blocking agents by producing a co-ordinated enhancement of multiple enzymes which detoxify carcinogens (14,15). This hypothesis is supported by the finding that C&K produce a marked enhancement of GST activity in the liver and small bowel of experimental animals (12,16,17). However, C&K may also alter carcinogen activation by the modulation of phase I xenobiotic metabolizing enzymes (CYP450) (18).

A previous study in our laboratory showed a marked dose-dependent increase in the expression and activity of GST-P in the liver of rats fed a diet containing C&K (17). In rats, GST-P is expressed in many extra-hepatic tissues, in foetal liver as well as in preneoplastic hepatic lesions (9,19–21). However, in the adult liver GST-P expression is essentially confined to bile duct cells and it is barely detectable in the hepatocytes of animals fed conventional laboratory chow diets (9). The effect of C&K on this GST isoenzyme is a transient hepatocellular induction. This is unrelated to the constitutive and irreversible expression of GST-P in foci which occurs as a result of cell transformation with carcinogens (17).

Several other potential chemoprotective agents such as dithiolethiones (22), ethoxyquin (23), butylated hydroxytoluene (BHT; 24) and butylated hydroxyanisole (BHA) (23,24) induce GST-P in a similar manner. Interestingly, these same compounds have been demonstrated to efficiently prevent AFB_1-

***Abbreviations:** $AFB_1/M_1/Q_1/P_1$, aflatoxin B1/M1/Q1/P1; AFBO, aflatoxin B1-8,9-epoxide; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; C&K, cafestol and kahweol; CYP450, cytochrome P450; D3T, 1,2-dithiole-3-thione; GSH, glutathione; GST, glutathione *S*-transferase.

induced hepatocarcinogenesis in the rat (2,25). The chemoprotective activity of these agents is believed to be due, at least partly, to an induction of an alpha class GST, named GST Yc2 (or rGSTA5) (25–28). Of all GSTs present in rat liver, this isoenzyme has been shown to possess the highest detoxifying activity toward AFBO (26,29). GST Yc2, like GST-P, is essentially absent in adult rat hepatocytes, but is expressed in developing liver as well as in carcinogen-induced hepatic nodules and hepatomas (9,29).

The similar inducibility of both GST Yp and Yc2, their comparable pattern of expression in the liver, and the previously reported effects of C&K on GST Yp expression prompted us to investigate whether C&K could induce the GST Yc2 subunit in rat liver. The consequences of such an induction, as well as the potential contribution of C&K-mediated modulation of phase I xenobiotic metabolizing enzymes to the overall chemoprotective effects of these coffee-specific components have been addressed.

Materials and methods

Treatment of animals

Five-week-old male Sprague–Dawley rats (Iffa Credo S.A., l'Arbresle, France) were used. They were provided with laboratory chow (Nafag 890, Nähr- and Futtermittel A.G., Gossau, Switzerland) and water *ad libitum* throughout the studies, which were performed under certified good laboratory practices (GLP). Following 11 days of acclimatization on basal diet, the animals were randomly assigned to five treatment groups (A–E). The test material was a mixture of C&K that was solubilized at different concentrations in a 50:50 mixture of corn and palm oils employed as a vehicle. Group A received the control diet, which comprised basal diet with 2.5% of the vehicle. Groups B–E received basal diet with 2.5% of the vehicle containing increasing amounts of C&K to give final concentrations in the diet of 92 (B), 460 (C), 2300 (D) and 6200 p.p.m. (E). Subsets of animals were killed after 5 (group A and D), 15 (group A and D), 28 (group A–E) and 90 days (group A–D) of treatment. Additional subsets of groups A and D, were subjected to a 1 month recovery period in which animals were fed control diet after the 90 day test period in order to examine the reversibility of any effects. Each group or group-subset comprised at least four animals. Following death, livers were stored at –80°C.

Test material

A mixture of C&K palmitates was prepared from coffee oil according to the procedure of Bertholet (30). The mixture contained cafestol:kahweol in the proportions 52.5:47.5 and its purity was >95%.

RNA analysis

Total RNA was isolated according to the method of Chomczynski and Sacchi (31). Northern and dot blot analyses were performed according to standard procedures (32). Following UV cross-linking, nylon filters were prehybridized and then hybridized with a γ-^{32}P-labelled oligonucleotide complementary to GST Yc2, CYP 2C11 and CYP 3A2 mRNA (33,34). Northern blot analyses were first performed to confirm the specificity of the hybridization conditions. Dot blots were then used for quantification. Relative specific mRNA contents were determined by scanning laser densitometry of autoradiographs and corrected to account for potential variations in gel loading. The total RNA loaded in each well was estimated by subsequent hybridization using a ^{32}P-labelled oligonucleotide probe specific for 18S ribosomal RNA.

Western blot analysis

S9, cytosolic and microsomal protein fractions were prepared from the liver of each animal according to standard methods (35,36). Protein contents were measured using the method of Bradford (37), BSA serving as a standard. Samples isolated from five independent animals were analysed through the western blot technique. The fractions of liver homogenates were separated by SDS–PAGE on 10% polyacrylamide gels (10 µg protein/lane) and electroblotted onto nitrocellulose membrane (35). The blots were incubated with rabbit polyclonal antibodies raised against rat CYP 2C11, CYP 3A2 and CYP 1A1 (Daiichi Pure Chemicals, Japan) or GST Yc2 (kindly donated by Professor J.Hayes, Dundee University). They were subsequently probed with a goat anti-rabbit secondary antibody conjugated to horseradish peroxidase. CYP450s and GST isoenzymes were detected indirectly by chemiluminescence using an ECL western blotting detection kit (Amersham Life Science, Buckinghamshire, UK).

Analysis of DNA binding

The activation and binding of AFB_1 to DNA in the presence of S9 or microsomal fractions was examined using an approach similar to that described by Hashim *et al*. (38) with some modifications. Liver S9 fractions (500 µg), were added to a reaction mix (pH 7.5) containing 5 mM $MgCl_2$, 0.65 mM β-NADPH, 1.97 µM AFB_1 and 500 µg calf thymus DNA. Following addition of 30 nM [^{3}H]AFB_1 (16 Ci/mmol) and 5 mM reduced GSH, the reaction mixtures were incubated for 30 min at 37°C. The reaction was stopped with 0.2% SDS and 1.5 M NaCl. Proteins were precipitated on ice and the samples were centrifuged at 5000 *g* for 20 min at 4°C. The amount of tritiated [^{3}H]AFB_1 bound to DNA present in the supernatant was measured in a scintillation counter after ethanol precipitation and washing. Similar experiments were also performed using microsomal fractions of rat liver (500 µg) in place of S9 fractions.

Results

A dramatic increase in GST Yc2 expression was observed at both mRNA and protein level in livers of rats treated with C&K. Western blot analysis showed little or no detectable GST Yc2 in hepatic cytosol from animals fed control diet. However, a dose-dependent increase in the Yc2 protein expression was clearly detected after 28 days of treatment with C&K at 460 (5-fold induction), 2300 (11-fold induction) and 6200 p.p.m. (16-fold induction) as shown in Figure 1. A similar dose-dependent effect was observed in livers obtained from rats after 90 days of C&K treatment (data not shown). In the immunoblots for GST Yc2, an upper band corresponding to GST Yc1 subunit is always detected due to the cross-reactivity of the antiserum with this subunit (39). Messenger RNA analysis confirmed the C&K-induction of GST Yc2 expression that was observed at the protein level. Dot blot analyses were performed using a highly specific oligomer cDNA for GST Yc2, employing hybridization conditions previously optimized with northern blot (28). Densitometric analysis of autoradiograms from the 28 day dose–response study demonstrated an induction of Yc2 mRNA expression in rats treated with 2300 and 6200 p.p.m. C&K in their diets (Figure 2).

The time-course and reversibility of the effects of C&K on Yc2 expression was studied to confirm a transcriptional activation of this gene rather than a permanent derepression of GST Yc2 expression. Western blot analysis revealed that dietary C&K at 2300 p.p.m. produced a maximal induction of hepatic Yc2 protein within 5 days of treatment (Figure 3), which was maintained throughout the whole 90 day treatment period. The effect was reversible following withdrawal of C&K treatment for 1 month, the expression of GST Yc2 decreasing to a level similar to that observed in control animals which were continually maintained on control diet containing no C&K (Figure 4).

The marked induction of hepatic Yc2 expression by C&K provides a rationale for the hypothesis that these coffee components may protect against AFB_1-mediated genotoxicity. This was tested using an *in vitro* DNA binding assay. In the first instance, the incorporation of tritiated metabolites [^{3}H]AFB_1 into calf thymus DNA was examined in the presence of liver S9 fractions from rats treated with C&K for 28 days. Dietary C&K produced a dose-dependent inhibition of AFB_1–DNA adduct formation as shown in Figure 5A. A statistically significant decrease in AFB_1 binding to DNA ($P < 0.05$) was observed in the presence of S9 fractions from rats treated with 2300 p.p.m. C&K and a maximum inhibition of 50% of the control value was achieved with 6200 p.p.m. of C&K. These results using S9 fractions reflect the consequences of a global effect of C&K on both hepatic phase I and phase II biotrans-

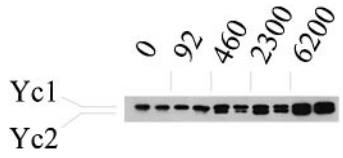

Fig. 1. Dose–response effect of C&K on the expression of hepatic GST alpha subunit Yc2 in Sprague Dawley rats. Cytosolic fractions (10 μg/slot) from livers of five independent rats fed either a control diet or diets containing C&K (92–6200 p.p.m.) for 28 days were analysed for the expression of the GST Yc2 subunit by western blot. Representative results using two or three different samples per treatment are presented. In the immunoblot for GST Yc2 (lower band), the antiserum shows cross-reactivity with the GST Yc1 subunit (upper band).

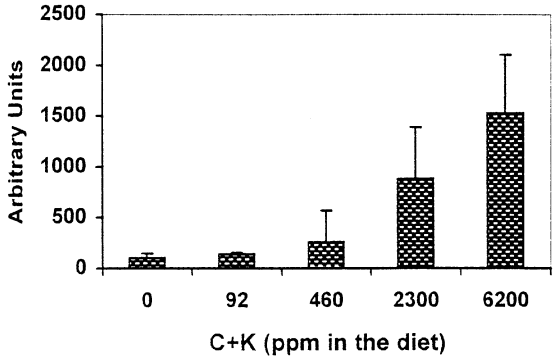

Fig. 2. Dose–response effect of C&K on the expression of hepatic GST Yc2 mRNA in Sprague–Dawley rats. Total RNA fractions extracted from the liver of four independent animals fed for 28 days with various amounts of C&K (0–6200 p.p.m.) were analysed by dot blot using an oligomer specific for GST Yc2 mRNA.

Fig. 3. Time-course of the effects of C&K on the expression of hepatic GST Yc2 in Sprague–Dawley rats. Western blot analysis were performed using cytosolic fractions (10 μg protein/lane) from the liver of control rats or rats treated with dietary 2300 p.p.m. of C&K for 5, 15, 28 or 90 days. Four independent animals per group were analysed, representative data are shown. In the immunoblot for GST Yc2 (lower band), the antiserum shows cross-reactivity with the GST Yc1 subunit (upper band).

Fig. 4. Reversibility of the effects of C&K on hepatic cytosolic GST Yc2 expression following a 1 month recovery period. Male rats were fed either a control diet or a diet containing 2300 p.p.m. of C&K for 90 days. After this test period, a subset of these animals was provided control diet for a month to examine the reversibility of the C&K effects (0 or 2300 R). Western blot analysis was performed using cytosolic fractions (10 μg protein/lane) from four independent rat livers, representative data are shown. Due to the cross-reactivity of the antiserum, two bands can be detected on the immunoblot, the upper band corresponds to the expression of the GST Yc1 subunit and the lower one to the GST Yc2 subunit expression.

formation enzymes involved in the activation and detoxification of AFB_1.

In order to examine whether the protective effects of C&K against AFB_1 may be mediated by events other than a modulation in Yc2 expression, the effects of liver microsomal

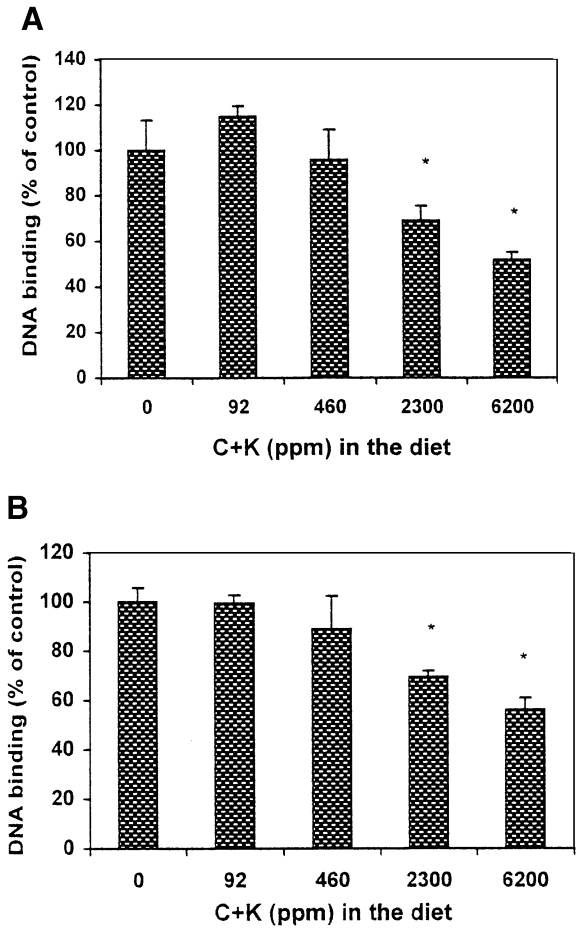

Fig. 5. Dose-dependent effect of C&K on the formation of AFB_1-induced DNA adducts *in vitro*. The incorporation of AFB_1 metabolites into calf thymus DNA were tested using liver S9 (**A**) or microsomal (**B**) fractions from male Sprague–Dawley rats fed either a control diet or diets containing C&K (92–6200 p.p.m.) for 28 days. Results presented are means obtained from at least three animals per group (±SEM). They are expressed as the percentage of the mean value derived from the control group. *Significantly different ($P < 0.05$) from animals fed control diet using the Student's *t*-test.

fractions from rats fed C&K for 28 days on AFB_1–DNA binding were investigated. A similar dose-dependent inhibition of binding was observed with the microsomal fractions (Figure 5B) as was previously seen with the S9 fractions. Liver microsomes from rats treated with C&K at a dose of 2300 p.p.m. produced a statistically significant ($P < 0.05$) inhibition of AFB_1–DNA binding and a 40% inhibition of binding was measured with microsomes from rats treated at the highest dose of C&K (6200 p.p.m.).

The effects of a comparison of the different liver fractions from animals fed C&K on AFB_1–DNA binding suggest that in addition to their effects on Yc2, C&K modulate phase I microsomal enzymes involved in the metabolism of AFB_1, resulting in a decreased formation of reactive metabolites. We therefore examined whether C&K could induce changes in hepatic enzymes involved in the activation of AFB_1 or in its transformation to a variety of reduced and oxidized derivatives (AFM_1, AFQ_1, AFP_1).

In the rat, the 8,9-epoxidation of AFB_1 is catalysed mainly by the male-specific CYP2C11 and CYP3A2 (25,28,40,41). Microsomal fractions of liver samples from the 28 day dose–response study were analysed by western blot using antibodies raised against rat CYP2C11 and CYP3A2. As shown in

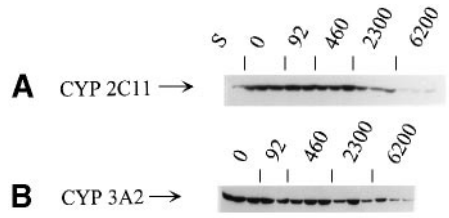

Fig. 6. Dose–response effect of C&K on the expression of hepatic cytochromes P450 CYP2C11 (**A**) and CYP3A2 (**B**) in male Sprague–Dawley rats. Western blot analyses were performed using 10 μg of microsomal protein from livers of five different animals fed either a control diet or diets containing C&K (92–6200 p.p.m.) for 28 days. Representative results are presented.

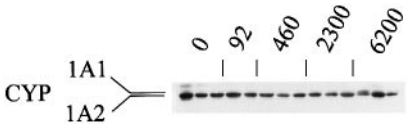

Fig. 7. Dose–response effect of C&K on the expression of hepatic cytochrome P450 CYP 1A1/1A2 in male Sprague–Dawley rats. Western blot analyses were performed using 10 μg of microsomal protein from livers of five different animals fed either a control diet or diets containing C&K (92–6200 p.p.m.) for 28 days. Representative results are presented.

Figure 6A, CYP2C11 protein expression level was decreased significantly by 35% at 2300 p.p.m. and 88% at 6200 p.p.m. of C&K in the diet as compared with control level. A dose-dependent reduction of CYP3A2 protein expression was also observed (40 and 60% inhibition at 2300 and 6200 p.p.m. of C&K in the diet, respectively) at this time point (Figure 6B). Treatment of the animals for 90 days with C&K resulted in similar inhibitions of CYP 2C11 and CYP 3A2 expression (data not shown).

Since in the western analyses, the normalization of the samples was based on total protein amount, the relative reduction in CYP2C11 and CYP3A2 protein expression could be the result of a large induction of other microsomal proteins. Such an hypothesis appears unlikely. Gels and blot membranes stained with Coomassie blue and Ponceau red, respectively, did not reveal any significant C&K-dependant alteration in the protein pattern (data not shown). Furthermore, no effects were found in the expression of other microsomal markers as assayed by western analysis [CYP1A1/1A2 (Figure 7); and CYP2C6 and CYP2E1 (data not shown)] or enzymatic methods (ethoxy and pentoxy *O*-deethylase activity, data not shown).

RNA analyses confirmed the inhibitory effect of C&K on the expression of specific cytochromes P450. A dose-dependent decrease in the expression of the mRNA coding for CYP2C11 and CYP3A2 was found which was maximal at 6200 p.p.m. of C&K [69% (Figure 8A) and 66% (Figure 8B), respectively] after 28 days of exposure to these components. Similar effects were observed following 90 days of C&K treatment (data not shown).

In the rat, the transformation of AFB_1 into AFM_1 by the CYP450s of the 1A subfamily may be considered as a detoxification reaction. To examine if C&K could modulate AFB1 genotoxicity via this pathway, western analysis for CYP1A1/1A2 was conducted. In both control and C&K treated animals, a constitutive expression of CYP1A2 was observed, while a weak band, or no band at all, corresponding to CYP1A1 was detected (Figure 7). No difference for either CYP1A1 or CYP1A2 expression was found as a result of 28 days of C&K treatment. The absence of C&K effects on this subfamily of

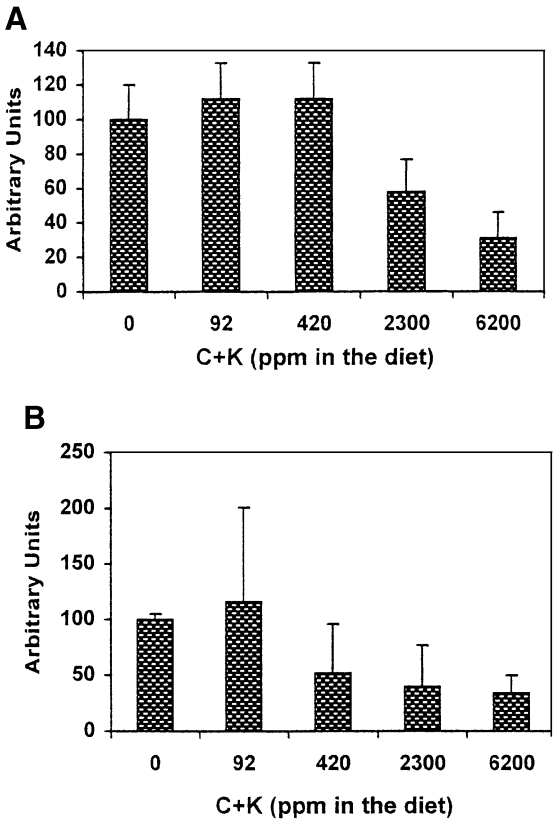

Fig. 8. Dose–response effect of cafestol and kahweol (C&K) on the expression of hepatic CYP3A2 and CYP2C11 mRNA in male Sprague–Dawley rats. Total RNA fractions extracted from the liver of four independent animals fed for 28 days with various amounts of C&K (0–6200 p.p.m.) were analysed by dot blot using oligomers specific for CYP2C11 (A), and CYP3A2 mRNA (B).

CYP450s was confirmed at the enzymatic level, by measuring the CYP1A-dependent ethoxyresorufin *O*-deethylase activity in microsomal liver fractions of rats fed C&K for 28 or 90 days (data not shown).

Discussion

The coffee-specific diterpenes C&K have been reported to be anticarcinogenic in several animal models (13,14,42). Although the mechanism involved has not yet been fully elucidated, experimental evidence strongly suggests that this chemoprotective activity may be related to the ability of C&K to induce GSTs (12,16). In the rat model, we have recently observed that the most striking effect of C&K was an hepatic induction of the placental GST subunit Yp (17), an isoform barely detectable in the liver of untreated adult animals, but highly expressed in foetal, neonatal and transformed hepatocytes (9,19,20). The similarity with respect to developmental expression pattern (29) and inducibility of GST Yp and Yc2 (22,23,24) prompted us to investigate the potential effect of C&K on the expression of this latter enzyme.

A marked dose-dependent induction of GST Yc2 protein was observed in the liver of rats fed a diet containing C&K. The parallel increase in the levels of specific Yc2 mRNA suggests a transcriptional activation of the GST Yc2 gene and/or an increase in Yc2 mRNA stability. The findings that the induction occurred within a short period of time (within 5 days) and was completely reversible, indicate that the effect of C&K on Yc2 is a transient induction which is unrelated to

the permanent derepression which may occur as a consequence of hepatocyte transformation.

An *in vitro* DNA-binding assay was applied to evaluate the potential chemoprotective activity of C&K against AFB_1 genotoxicity resulting from the elevated Yc2 expression. Liver S9 prepared from animals fed a diet containing C&K at levels of 2300 p.p.m. and higher prevented the covalent binding of AFB_1 to DNA. A similar inhibition of the AFB_1–DNA binding was observed using microsomal fractions instead of the S9 fractions. Overall, these data are compatible with a net protective effect of C&K against the genotoxicity of AFB_1. Furthermore, they suggest a complex mechanism involving both phase I and phase II metabolizing enzymes.

Several phase I-mediated mechanisms may potentially be responsible for a protective effect of C&K against AFB_1 genotoxicity. A C&K-dependent inhibition of the expression of the phase I AFB_1-activating systems, which would reduce the cellular production of the reactive metabolite AFBO, was the first hypothesis tested. It is currently thought that AFBO is primarily produced in the liver through CYP450-mediated reactions. Two specific forms of CYP450, CYP2C11 and CYP3A2, have been implicated in the epoxidation of AFB_1 to AFBO in male rats (25,39,43,44). Western blot analysis indicated that C&K decreased the protein expression of both CYP2C11 and CYP3A2. RNA analysis using highly specific oligomers confirmed the reduction of CYP2C11 and CYP3A2 gene expression in C&K-treated animals. With respect to production of AFBO, the most genotoxic metabolite of AFB_1, the consequence of the C&K-dependent decrease in CYP2C11 and CYP3A2 expression is reflected in the DNA-binding assay data. AFBO is highly unstable and reacts rapidly with biological constituents. Its formation can be inferred by interception with trapping agents such as Tris or DNA. Therefore, the data obtained with the binding assay indicate that the decrease in enzyme expression results in a reduction of the production of AFBO.

In addition to epoxidation, AFB_1 may be transformed by the microsomal mono-oxygenase system into hydroxylated products such as AFM_1, AFQ_1 and AFP_1 (4,5,25,45). Since these metabolites possess low mutagenic potency as compared with AFBO, these particular phase I reactions may be considered as detoxification pathways. In the rat, the oxidation of AFB_1 into AFM_1 and AFQ_1 are catalysed by members of the CYP450 1A and 3A, respectively (4,5). In C&K-treated rats, the expression of CYP3A and CYP1A members were respectively reduced or not altered. These data, which were confirmed at enzymatic level (data not shown), indicate that the stimulation of the production of less genotoxic AFB_1 metabolites, such as AFM_1 and AFQ_1, is unlikely to be a relevant mechanism for the phase I-dependent protective action of C&K.

A direct C&K-dependent inhibition of the enzymatic activities responsible for the AFB_1 activation is another possible mechanism for phase I-mediated chemoprotection. However, adding C&K to the reaction mix in *in vitro* DNA-binding experiments failed to prevent the production of AFB_1 adducts formed in presence of microsomal fractions from untreated rat liver (data not shown).

The question arises whether chemical-induced anti-carcinogenic effects against AFB_1 in rats is of relevance with respect to human chemoprotection. In rats, the major detoxification mechanism for AFBO is by conjugation with GSH, catalysed by GSTs, and it has been shown that the induction of GST Yc2 by 1,2-dithiole-3-thione (D3T) in liver, contributed highly to the GSH conjugation of exo-AFBO (22,28). This isoenzyme is not constitutively expressed in the adult hepatocytes while it is highly inducible by compounds which inhibit AFB_1-induced hepatocarcinogenicity such as oltipraz, ethoxyquin, BHA and BHT (22,29,46,47). The AFB_1-conjugating activity of GST subunits in human liver is low (28,43). At the present time there is no evidence of the existence of a human GST isoenzyme presenting similar catalysing properties toward AFBO as the rat GST Yc2. Therefore, the relevance to humans of any induction of GST Yc2 in the rat is questionable, although the possibility that a non-constitutive, but inducible human form of GST with high AFBO-conjugating activity exists cannot be completely excluded (28).

The inhibition of the AFB_1-activating enzyme expression provides an alternative mechanism by which chemoprotective agents may reduce intracellular levels of AFBO. Oltipraz, a synthetic derivative of the natural 1,2-dithiole-3-thione, is a potent inhibitor of AFB_1-induced hepatocarcinogenesis in rat (46,48–50) and it has been selected for a chemoprevention trial in a Chinese population characterized by a high AFB_1-exposure (51,52). In rats, oltipraz, like C&K, may act through two distinct mechanisms, an induction of GST Yc2 (22,33) and an inhibition of CYP450-mediated AFB_1 activation (50,53,54). It has been recently shown that oltipraz does not stimulate GSH conjugation of AFB_1 in human primary hepatocyte cultures (55). However, oltipraz significantly inhibited the expression of phase I AFB_1-activating enzymes in this model. Thus, this latter effect appears to be a promising mechanism of oltipraz chemoprotection in humans. The similarity of the biochemical effects produced by C&K on AFB_1-metabolizing enzymes with those reported for oltipraz reinforces the idea that C&K consumption may confer a protection against AFB_1-carcinogenicity in humans.

In conclusion, the data presented are consistent with a potential protective effect of C&K against the hepatocarcinogenicity of AFB_1. Two complementary mechanisms may account for this chemoprotective activity. A decrease in the expression of AFB_1-activating enzymes as well as an increase in the expression of the GST sub-unit known to possess the highest catalytic activity toward the reactive metabolite AFBO. Importantly, the xenobiotic metabolizing enzymes modulated by C&K are well documented to possess the capability to activate other potential carcinogens or toxicants, and therefore chemoprotective effects against other compounds may be expected. For example, experimental data have indicated that CYP2C11 is involved in the bioactivation of several pro-carcinogens such as styrene (56) and 4-aminobiphenyl (57). Furthermore, 2C11 is known to metabolize the anti-inflamatory drug diclofenac into a highly reactive hepatotoxic product (58). Although the relevance of these data to the human situation remains to be demonstrated, they confirm previous findings (17,42,55) suggesting that these natural coffee-specific components may possess a broad range of promising chemoprotective properties.

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