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Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity

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Abstract

Epidemiological studies have found an inverse association between coffee consumption and the risk of certain types of cancers such as colorectal cancers. Animal data support such a chemopreventive effect of coffee. Substantial research has been devoted to the identification of coffee components that may be responsible for these beneficial effects. In animal models and cell culture systems, the coffee diterpenes cafestol and kahweol (C + K) were shown to produce a broad range of biochemical effects resulting in a reduction of the genotoxicity of several carcinogens including 7,12-dimethylbenz[*a*]anthracene (DMBA), aflatoxin B_1 (AFB_1), benzo[*a*]pyrene (B[*a*]P) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Different mechanisms appear to be involved in these chemoprotective effects: an induction of conjugating enzymes (e.g. glutathione *S*-transferases, glucuronosyl *S*-transferases), an increased expression of proteins involved in cellular antioxidant defense (e.g. γ-glutamyl cysteine synthetase and heme oxygenase-1) and an inhibition of the expression and/or activity of cytochromes P450 involved in carcinogen activation (e.g. CYP2C11, CYP3A2). In animal models, the C + K-mediated induction of conjugating and antioxidant enzymes has been observed in hepatic, intestinal and kidney tissues. In the small intestine, these inductions were shown to be mediated by Nrf2-dependent transcriptional activation. In vitro investigations obtained in cell cultures of human origin indicate that the effects and mechanisms observed in animal test systems with C + K are likely to be of relevance for humans. In human liver epithelial cell lines transfected to express AFB_1-activating P450s, C + K treatment resulted in a reduction of AFB_1–DNA binding. This protection was correlated with an induction of GST-mu, an enzyme known to be involved in AFB_1 detoxification. In addition, C + K was found to inhibit P450 2B6, one of the human enzymes responsible for AFB_1 activation. Altogether, the data on the biological effects of C + K provide a plausible hypothesis to explain some of the anticarcinogenic effects of coffee observed in human epidemiological studies and in animal experiments.

Keywords: Cafestol and kahweol; Coffee; Diterpenes; Chemoprotection; Mechanisms; Blocking agents; Glutathione *S*-transferases; Cytochromes P450

1. Introduction

Numerous epidemiological studies have investigated the relationship between coffee consumption and cancer incidence at various sites. Overall, there is no evidence that moderate (2–5 cups a day) coffee drinking represents a significant risk for the development of cancer in humans (Schilter et al., 2001a). In contrast, many studies have revealed an inverse (protective) association between coffee consumption and the risk of certain cancers (Nishi et al., 1996; Giovannucci, 1998; Inoue et al., 1998). The epidemiology of colorectal cancer provides the most supportive evidence of a potential coffee-dependent protection. In a recent meta-analysis comprising five cohort and 12 case-control studies, a significant inverse association between coffee consumption and colorectal cancer was found (Giovannucci, 1998).

Animal studies have provided further support for a potentially chemoprotective effect of coffee. In chronic studies conducted in rodents, coffee administered at high levels in the diet resulted in a decreased incidence of spontaneous tumors at different organ sites (Würzner et al., 1977; Stalder et al., 1990). Other studies have shown that coffee or coffee constituents protect against

Abbreviations: AFB_1, aflatoxin B_1; AFBO, AFB_1-8,9-epoxide; B[*a*]P, benzo[*a*]pyrene; C + K, CDNB, chlorodinitrobenzene; DMBA, dimethylbenz[*a*]anthracene; GCS, γ-glutamyl cysteine synthetase; GSH, reduced glutathione; GST, glutathione *S*-transferase; HO-1, heme oxygenase 1; HSP, heat shock protein; NQO, NAD(P)H:quinone reductase; P450, cytochrome P450; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

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the action of well-known carcinogens such as nitrosamines (Nishikawa et al., 1986) or 1,2-dimethylhydrazine (Gershbein, 1994). Several studies have demonstrated that green as well as roasted coffees inhibit the development of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced carcinogenesis at various tissue sites in different experimental animal cancer models (Wattenberg, 1983; Miller et al., 1988, 1993).

A number of coffee components have been identified as being potentially responsible for the chemoprotective effects of coffee. Among others, caffeine (Rothwell, 1974) and polyphenols including chlorogenic acids and their degradation products have been considered as good candidates (Stadler, in press; Schilter et al., 2001b). In addition, investigations performed in rats, mice and hamsters led to the identification of a specific lipidic fraction as potentially responsible for the chemopreventive effects of coffee on DMBA-induced cancer (Lam et al., 1982; Wattenberg et al., 1986, Miller et al., 1991). The major constituents of this fraction were found to be the diterpenes cafestol and kahweol (C + K; Fig. 1). These specific coffee constituents are very difficult to isolate independently and kahweol is highly unstable when purified. Therefore the biological properties of these compounds have been studied traditionally using a mixture of both. For the experiments conducted in the laboratories of the present authors, C + K mixture (purity > 98%) was prepared from coffee oil in a ratio of 52.5 (C):47.5 (K) (Bertholet, 1987). In the following sections, recent data supporting the potential protective role of C + K in animals and humans are provided. In addition, mechanisms of actions are discussed.

2. Alteration of carcinogen–DNA binding

In addition to its protective effects against the development of DMBA-induced tumors, C + K was shown to prevent the formation of DNA adducts of several genotoxic carcinogens. In an in vitro DNA-binding assay conducted in the presence of liver S9 fractions from rats treated over 28 days with diets containing C + K at levels ranging from 0 to 6200 ppm, a significant dose-dependent decrease in the incorporation of tritiated metabolites of the hepatocarcinogenic mycotoxin aflatoxin B_1 (AFB_1) into calf thymus DNA was found (Cavin et al., 1998). Similar S9 fractions from the same feeding study were used in the metabolic activation mix of a bacterial mutation assay. Although a substantial variation was observed between rats within each group, significant reduction ($P = 0.015$) of the number of revertants induced by AFB_1 was observed with the liver S9 fractions from the rats fed diets containing the highest doses of C + K (2300 and 6200 ppm) (Fig. 2). This gave a further indication that dietary C + K may reduce the genotoxicity of AFB_1. Data generated in primary cultures of freshly isolated rat hepatocytes support the results obtained ex vivo. The pre-treatment of these cells with C + K (0–4 μg/ml) resulted in a dose-dependent inhibition of AFB_1-induced DNA adduct formation (Cavin et al., 2001; Fig. 3) . In addition, such a treatment was shown to also prevent the DNA-binding of benzo[*a*]pyrene (B[*a*]P), an ubiquitous environmental carcinogen of the polycyclic aromatic hydrocarbon class (Fig. 3).

A C + K-mediated prevention of DNA binding was also observed in extrahepatic tissue with the procarcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). PhIP, a heterocyclic amine to which humans are strongly exposed under real-life conditions, is a pyrolysis product found in cooked meat and fish, thus also being described as "cooked food mutagen". In the rodent, PhIP was found to produce cancers of breast, prostate and lymphatic system and, most importantly, of the colon. In agreement with this, PhIP has been implicated in the etiology of human colon cancer. Male F344 rats fed a diet containing 2000 ppm

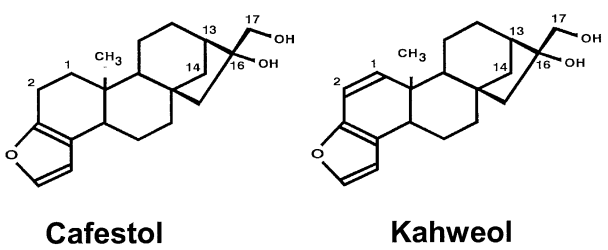

Fig. 1. The coffee specific diterpenes cafestol and kahweol: chemical structure.

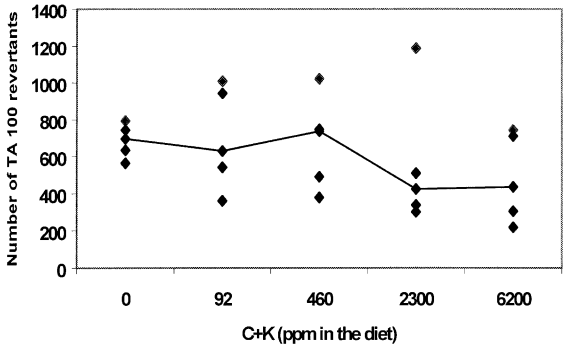

Fig. 2. Effects of C + K on the mutagenic response to aflatoxin B_1 (AFB_1) in the Ames test. S9 fractions (0.6 mg/plate prepared according to Schilter et al., 1996) from livers of male rats fed either a control diet or diets containing C + K (92–6200 ppm) for 28 days were mixed with 2 nM AFB_1 and strain TA100, plated directly and incubated for 3 days at 37 °C. Spontaneous revertants numbers for TA100 were 122 ± 17. Line represents the median values of each independent test group of five rats. Each rat liver S9 was tested in triplicate (variability was within 10% of the average value). Reduction of AFB_1 induction of revertants was statistically significant ($P = 0.015$) with liver fractions from rat fed with 2300 and 6200 ppm C + K in the diet using the *p* (Fisher's exact) test.

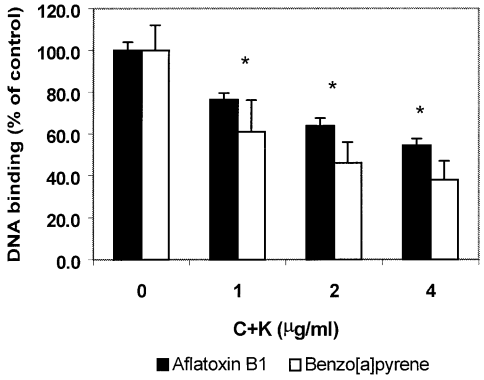

Fig. 3. Dose-dependent effect of cafestol and kahweol (C + K) on the formation of AFB_1 and B[*a*]P-induced DNA adducts in vitro. The incorporation of 20 nM tritiated [^{3}H]AFB_1 metabolites into DNA and 0.5 μM [^{3}H]B[*a*]P were tested using rat primary hepatocytes pretreated with increasing concentrations of C + K (0–4 μg/ml) for 48 h. Primary rat hepatocyte cultures were prepared according to Cavin et al. (2001). Results presented are means obtained from five experiments with two independent cultures per treatment (±S.D.). These are expressed as the percentage of the mean value derived from control cultures. In controls, the absolute binding rate (equal to 100%) were in average 6.5 pmol AFB_1 and 4.5 pmol B[*a*]P/mg DNA, respectively. *Significantly different from control hepatocytes ($P < 0.05$) using the Student's *t*-test.

C + K for 10 days received 50 mg/kg PhIP through gavage. After 24 h, PhIP–DNA adducts were analyzed in the colon and the PhIP–DNA adduct formation was significantly lower in C + K treated animals than in the controls (Huber et al., 1997). Altogether, these data indicate that C + K may prevent DNA damage induced by a wide range of carcinogens and these protective effects may occur in various tissues.

3. Chemoprotective mechanisms

The mechanisms responsible for the chemoprotective effects of C + K are not fully understood. Chemopreventive agents may intervene at one or several steps of the carcinogenic process such as during the initiation, promotion or progression stages (De Flora and Ramel, 1988; Harris, 1991). It is known that the initiation of tumor formation, which generally consists of a permanent modification of DNA with electrophilic or oxidant metabolites derived from procarcinogen biotransformation, is a target for several dietary anticarcinogenic compounds, the so-called blocking agents (Wattenberg, 1985). They act through an inhibition of the formation and/or the stimulation of the detoxification of the electrophilic or oxidant intermediates, resulting in decreased DNA damage and in the blocking of initiation. Early studies indicated that C + K induced glutathione *S*-transferase (GST) activity in mouse liver and small intestine (Lam et al., 1982). Since GST is known to detoxify electrophilic compounds through conjugation with glutathione, these data led to the hypothesis that C + K may possess the properties of blocking agents. Recent studies further confirm this hypothesis and show that C + K-preventive effects may be mediated by both an inhibition of bioactivation and a stimulation of detoxification.

3.1. Phase II-mediated mechanisms

3.1.1. Induction of phase II detoxifying enzymes

Following up on the early observations showing an induction of GST-mu in mouse liver and small bowel (Lam et al., 1982), the effects of C + K on the expression of various GST subunits were studied in the rat (Schilter et al., 1996; Cavin et al., 1998), the animal species in which the anticarcinogenic properties of these compounds were first demonstrated. Rats were administered C + K in the diet (0, 92, 460, 2300, 6200 ppm) over periods of up to 90 days. The most striking effects identified were a strong dose-dependent induction of the GST Pi subunit Yp and the alpha subunit Yc2 (rGST A5) in the liver following 28 or 90 days of treatment. The effects were found at the mRNA and protein levels and were significant ($P < 0.05$) at C + K dietary concentrations of 460 ppm and higher. With respect to GST-P, time-course experiments indicated that the C + K-mediated induction occurred within a few days. In addition, it was shown that the increased expression was dependant on the continuous presence of C + K in the diet and was reversible following removal of C + K (Schilter et al., 1996). In agreement with this, levels of overall GST activity, as measured with chlorodinitrobenzene (CDNB), increased two- to three-fold during a 10-day dietary exposure to 2000 ppm C + K, but returned to pretreatment levels 10 days after removal of the treatment diet (Huber et al., 1998). Immunohistochemical examination of liver slices revealed that the GST-P induction occurred predominantly in periportal hepatocytes (Schilter et al., 1996). The contribution of these effects on GST expression to the chemoprotective properties of C + K is difficult to predict, due to the low content of this isozyme in control livers. However, it is important to keep in mind that GST-P has been implicated in the detoxification of both B[*a*]P and PhIP reactive metabolites (Swedmark et al., 1992; Fields et al., 1994; Lin et al., 1994). Furthermore, GST Yc2 is known to efficiently conjugate aflatoxin B_1-8,9-epoxide (AFBO), the most genotoxic metabolite of AFB_1 (Hayes et al., 1991; Pulford and Hayes, 1996).

The effects of C + K on the expression of GST enzymes were also investigated in the small intestine of mice fed 250 ppm dietary C + K for 14 days (McMahon et al., 2001). As previously found in rat liver, exposure to C + K resulted in a significant induction in GST general activity (2.5-fold) in the small intestine. Furthermore, Western analysis applying GST specific

antibodies revealed a two- to three-fold increase in the intestinal expression of several alpha (GST A1/2, A3, A4) and mu (GST M1, M5) GST subunits. Preliminary experiments from our laboratories suggest that similar induction of alpha and mu GST subunits may also occur in the colon of rats treated with C+K (C. Cavin, unpublished data), while in the liver hepatic θ activity appeared to be increased as well (Huber et al., 2000). Considerable organ-specific differences were observed regarding the effects of C+K on overall GST activity using CDNB as substrate. In rats fed 0.2% C+K, strong increases in the liver and kidneys (two- to three-fold as compared to controls) were paralleled by a moderate enhancement in the lung, a marginal one in the colon, and no changes in several other organs such as pancreas, salivary gland and testis (Schilter et al., 1996; Huber et al., 1997, 2000; W.W. Huber et al., unpublished). The liver and other well-perfused organs may thus contribute to chemoprotective effects that occur in distant organs as well.

In addition to the effects on GST expression, C+K were found to strongly induce several other phase II xenobiotic metabolizing enzymes. A dose-dependent increase in glucuronosyl *S*-transferase (UDP-GT) activity was recently observed in the liver and kidney of rats fed a diet containing between 120 and 1200 ppm of C+K (free alcohols) for 10 days. (Huber et al., 2000). Dietary exposure of mice to 250 ppm C+K for 14 days also showed a significant induction of NAD(P)H:quinone oxidoreductase activity in their small intestine of about two-fold (McMahon et al., 2001).

3.1.2. Molecular mechanism of induction

The *cis*-acting antioxidant-responsive-element (ARE) sequence has been identified on the promoter of several genes involved in detoxification processes (Hayes et al., 1999). It has been suggested that altering the expression of these genes through ARE-mediated transcriptional activation is likely to be a key molecular mechanism explaining how many blocking agents may prevent mutagenesis. Uncertainty exists about the identity of the transcription factor(s) that mediate ARE-driven gene induction. Recently, bZip Nrf proteins have been found to activate gene induction through this specific enhancer (Venugopal and Jaiswal, 1996). The role of Nrf2 transcription factor in the C+K-mediated activation of intestinal detoxifying enzymes has been addressed using a mouse line bearing a targeted disruption of the gene encoding this factor (McMahon et al., 2001). As mentioned previously, both NQO and GST enzymes activities were induced in the small intestine of the wild-type animals treated with C+K for 14 days, whereas such induction could not be observed in mutant knockout mice. These results demonstrate the key role of this transcription factor in the chemoprotective activity of C+K in the small intestine. Nrf2 is significantly expressed in the liver, suggesting that a similar role may also be expected in this organ.

3.2. Phase I-mediated mechanisms

3.2.1. Inhibition of phase I activating enzyme expression

Beside a stimulation of detoxification processes, a reduction of carcinogen activation was shown to play an important role in the C+K-mediated prevention of carcinogen DNA binding. It was observed that in rats fed diets containing C+K over 28 days, the hepatic expression of the cytochrome P450 (P450) CYP3A2 was significantly decreased at both mRNA and protein levels (Cavin et al., 1998). Significant differences as compared to controls were found at dietary C+K concentrations of 2300 ppm and above. The effects on protein expression were correlated with a decrease in 3A-dependent enzymatic activity (Fig. 4). Other P450 were altered by C+K treatments. For example, the expression of the male-specific P450 CYP2C11 was also significantly decreased by 35% at 2300 ppm and 88% at 6200 ppm of C+K in the diet as compared with control level (Cavin et al., 1998). These effects on CYP3A2 and CYP2C11 were confirmed in rat primary hepatocyte cultures. Since CYP2C11 and CYP3A2 are the major P450s responsible for the bioactivation of AFB_1 into AFBO in the rat, it was hypothesized that a reduction in the expression of these genes may contribute to the C+K-mediated prevention of AFB_1–DNA adducts (Forrester et al., 1990). In a DNA-binding assay, the use of microsomes from the liver of C+K treated rats as activating system resulted in a significant reduction of AFB_1–DNA adducts formation (Cavin et al., 1998). This effect was dose dependent and maximal at 6200 ppm in the diet (40% reduction). These data support a role for the decrease in phase I enzyme expression in the

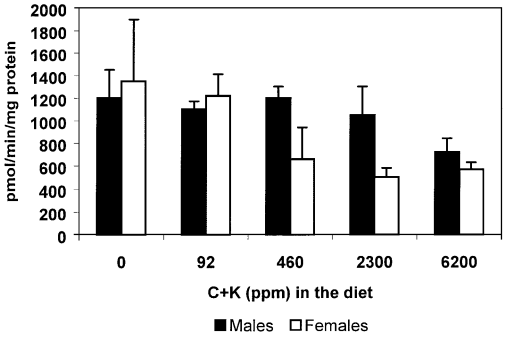

Fig. 4. Dose–response effect of cafestol and kahweol (C+K) on the activity of hepatic cytochrome P450 3A in rats. The P450 CYP 3A-mediated activity of liver microsomal fractions of male and female rats fed either a control diet or diets containing C+K (92–6200 ppm) for 28 days was assayed according to Wrighton et al. (1986), using erythromycin as a substrate. Results presented are means obtained from five animals per group with two individual measurements per animal (±S.D.).

chemoprotective effects of C + K against AFB_1 genotoxicity.

3.2.2. Inhibition of phase I enzymatic activity

A direct inhibition of P450 enzymatic activity without any effects on protein expression is an additional phase I-mediated mechanism through which the coffee diterpenes may act. For example, C + K has been shown to produce an inhibition of P4501A1 activity in liver cells which resulted in a reduction of B[*a*]P activation and DNA binding (C. Cavin, unpublished data). Similar inhibitory effects were found with human P450 CYP 2B6, a human P450 responsible for AFB_1 bioactivation (Cavin et al., 2001). In addition, it is suggested that the reduction of PhIP–DNA adducts found in C + K-treated rats involves an inhibition of the PhIP-activating enzymes P450 1A2 and *N*-acetyl transferase (NAT) (Huber et al., 1998, 2000). C + K was also found to reduce the *N*-hydroxylation of 4-aminobiphenyl in vitro (Hammons et al., 1999).

3.3. Antioxidant-dependent mechanisms

Cis-acting AREs have been described not only in promoters of conjugating enzymes but also in promoters of genes encoding enzymes involved in cellular antioxidant defense such as the catalytic heavy subunit of γ-glutamyl cysteine synthetase (GCS) and heme oxygenase-1 (HO-1) (Prestera et al., 1995; Mulcahy et al., 1997). There is increasing evidence that oxidative damage is involved in various pathological processes such as cancer, therefore the increase in antioxidant enzyme expression may be of significant importance in chemoprevention.

GCS is the rate-limiting enzyme in the synthesis of the tripeptide glutathione (GSH). GSH is a major intracellular antioxidant which plays an important role in maintaining cellular redox status and in the detoxification of activated xenobiotics. In rat primary hepatocyte cultures, C + K produced a dose-dependent increase in the intracellular GSH concentration (Fig. 5), This effect is likely to reflect an Nrf2-mediated transcriptional activation of GCS, as described in the mouse intestine. Indeed, inductions of GCS were found upon C + K treatment in liver, kidney, colon and lung of rats which were associated with increases in the GSH content of the same organs (Huber et al., 2000).

HO-1 is a protein belonging to the cellular stress response. It is rapidly induced by oxidative stress related stimuli such as glutathione depletion, heavy metal exposure and oxidative damage. HO-1 is known to play a role in free radical scavenging. As expected from the data available on Nrf2-mediated gene regulation (Alam et al., 1999), a marked dose-dependent induction of HO-1 protein expression was obtained in primary rat hepatocytes as a result of C + K treatment (Fig. 6A).

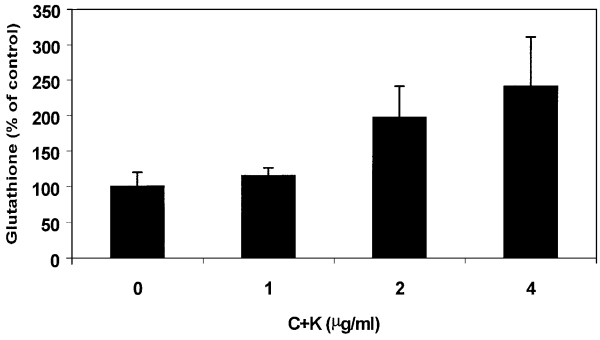

Fig. 5. Dose–response effect of cafestol and kahweol (C + K) on the glutathione (GSH) content of rat primary hepatocytes. Primary rat hepatocyte cultures were prepared according to Cavin et al. (2001). The GSH level of control and C + K (1–4 μg/ml)-treated hepatocytes for 48 h was assayed according to Gallagher et al. (1994). Results presented are means obtained from at least three experiments with two independent cultures per treatment (±S.D.). In control hepatocytes, the GSH activity was in average 30 nmol/min/mg protein.

Further data have indicated that the diterpenes may induce other genes of the cellular stress response. A significant induction of heat shock protein 25 (HSP25) expression was found in rat hepatocytes following 48 h of C + K exposure treatment (Fig. 6B). This chaperone is known to carry out important functions in protein synthesis, maturation and translocation across membrane (Ehrnsperger et al., 1998). It is an element of the adaptive response to environmental stresses and pathophysiological states (inflammation, viral and bacterial infection, aging, etc.). It is involved in recognizing injured proteins and participates to the process of protein repair and elimination.

4. Relevance to humans

4.1. C + K effects in human test systems

The potential chemoprotective activity of C + K in human is still under investigation. The application of human liver epithelial cell lines (THLE) stably transfected to express human AFB_1-activating P450s including CYP 1A2, CYP 3A4 and CYP 2B6 indicate that C + K is likely to produce in human cells similar effects to those observed in animal models (Cavin et al., 2001). In these cells, C + K treatment for 48 h significantly decreased AFB_1–DNA adduct formation. Maximum inhibitions of 30% (T5–2B6) and 45% (T5–1A2) as compared to control cells were achieved at a C + K concentration of 5 μg/ml. This preventive effect was correlated with an increase in the general GST activity together with a marked dose-dependent induction of GST-mu (2.5-fold), which was maximal after 48 h of treatment with C + K. Human GST-mu is known to be involved in AFBO detoxification (Langouët et al., 1998).

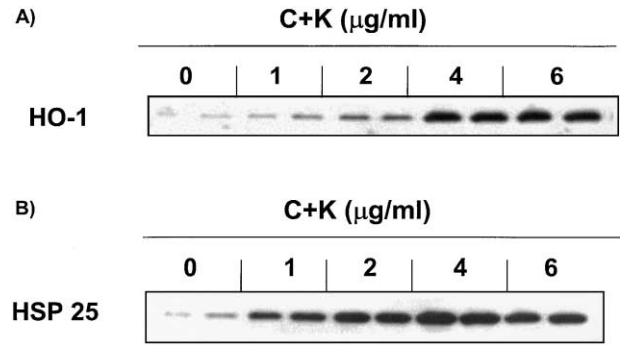

Fig. 6. Representative dose–response effect of cafestol and kahweol (C+K) on the expression of heme oxygenase I (HO-I) (A) and heat shock protein 25 (HSP 25) (B) in rat primary hepatocytes. Primary rat hepatocyte cultures were prepared according to Cavin et al. (2001). Western blot analysis was performed using 25 μg of whole protein fractions from control or C+K (1–6 μg/ml)-treated hepatocytes for 48 h.

The possibility of a direct inhibition of the enzymatic activity responsible for AFB_1 activation was tested in the different P450 expressing THLE clones. A selective C+K-dependent inhibitory effect (60% at 5 μg/ml) was found in THLE-2B6 (Cavin et al., 2001). In addition, preliminary data in human primary hepatocytes suggest that the coffee diterpenes may significantly inhibit the expression of P450s involved in AFB_1 activation at a concentration as low as 1 μg/ml (S. Langouët, personal communication). The data obtained using AFB_1 as a model indicate that the effects and mechanisms obtained with C+K in animal test systems are likely to be of relevance for humans. Recent data generated in our laboratory using other carcinogens such as B[*a*]P support this hypothesis. Further preliminary results suggest that C+K is also capable of enhancing GCS and GSH in HepG2 cells, an other commonly used human-derived hepatic cell line (Scharf et al., 2001). With respect to drug metabolizing enzymes, the data suggest that C+K produce effects in human cells similar to those observed in animal models (Cavin et al., 2001).

4.2. Significance of the C+K-mediated effects in relation to coffee consumption

Coffee drinking is the major source of human exposure to C+K. An important question regarding the health significance of the biological activity of C+K is to know whether the effects obtained with very high doses in animals may occur in humans consuming moderate amounts of coffee (up to 5 cups a day). Previous data showing an increased salivary content of GST in individuals consuming large amounts of coffee (Sreerama et al., 1995) suggest a possible coffee-dependent induction of these enzymes in human in vivo. However, the role of C+K in these effects has not been clarified.

The human exposure to C+K is highly dependent on the type of brew consumed. Chemical analysis of the diterpenes in different coffee brews has shown that Scandinavian-type boiled coffee, Turkish and cafetière coffee contain the highest amounts of C+K, while instant and filtered coffee contain negligible amounts and expresso intermediate amounts (Urgert et al., 1995). In epidemiological studies, the consumption of boiled coffee was associated with an increase in blood cholesterol levels (Thelle et al., 1987; Urgert and Katan, 1997). Clinical studies identified C+K as the causative factors of this effect (Weusten-van der Wouw et al., 1994; Urgert and Katan, 1997). These investigations demonstrated a dose-dependent and reversible effect of C+K on blood cholesterol. Based on these clinical and epidemiological studies, it was estimated that an exposure to 10 mg of total diterpenes per day is likely to have no or negligible hypercholesterolemic effect (Urgert et al., 1995; Urgert and Katan, 1997). Taking into consideration the occurrence of the diterpenes in various coffee brews indicates that except for boiled coffee, moderate coffee consumption is highly unlikely to alter blood cholesterol levels. In this context, the question remains as to whether safe levels of diterpene exposure through common coffee consumption may produce beneficial health effects.

Available information on the effects of C+K in vivo and in vitro may provide some preliminary answers to the above question. C+K doses required to induce effects on xenobiotic metabolizing enzymes have been found to be similar in cell cultures of both rat and human origin. This suggests that differences in response to C+K between human and rat are most probably at the kinetic rather than dynamic level. There are presently no kinetic data available on C+K that would allow adequate comparisons between rats and humans. Since the hypercholesterolemic effect has been well characterized in both rat and humans, such a parameter may serve as a marker of diterpene internal doses. In our studies investigating the biological effects of C+K in the rat, a significant increase in blood cholesterol was observed at the highest dose only (6200 ppm in the diet) (Fig. 7B). Importantly, effects compatible with chemoprotection were usually observed at doses significantly lower. This is illustrated by the GST Yc2 and Yp protein induction which was significant at a dietary C+K of 460 ppm (Fig. 7A). In another rat study, hepatic induction of UDP-GT activity (50%) was observed at a level as low as 120 ppm of C+K (Huber et al., 2000). This treatment did not produce any hypercholesterolemia (W.W. Huber, personal communication). Assuming that the difference between the dose–response relationship for the hypercholesterolemic and the enzyme induction effects may be similar in humans and rat, beneficial effects in humans might be expected at C+K levels that do not produce any increase in blood cholesterol.

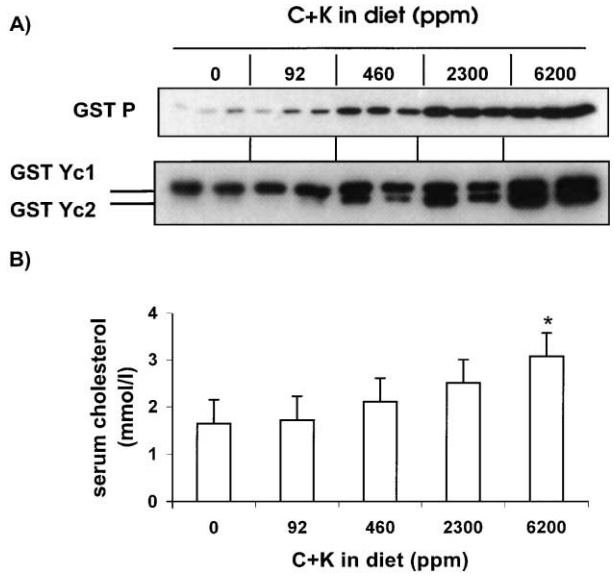

Fig. 7. Dose–response effects of cafestol and kahweol (C + K) on the expression of hepatic detoxifying enzymes (GST Yp and Yc2) (A) and on plasma cholesterol levels (B) in rats. Cytosolic fractions from liver of rats fed either a control diet or diets containing C + K (92–6200 ppm) for 28 days were assayed for the expression of GST Yp and GST Yc2 by western blot according to Schilter et al. (1996) (A) Total plasma cholesterol levels were analysed on a Cobas Mira apparatus (Roche), using an enzymatic assay (Cholestérol RTU kit, bioMérieux). Cholesterol values represent the mean ±S.D. ($n = 10$) (B). *Significantly different from controls ($P < 0.05$) using the Student's t-test.

5. Conclusion

In animal models including hamster, rat and mouse, the coffee-specific diterpene cafestol and kahweol have been shown to produce biological effects compatible with anticarcinogenic properties. They have been shown to occur in liver, kidney, lung and intestinal tissues. The use of several model carcinogens led to the identification of several key mechanisms:

- An induction of phase II enzymes involved in carcinogen detoxification.
- A reduction in the expression of phase I enzyme responsible for carcinogen activation.
- A specific inhibition of the enzymatic activity of phase I enzyme responsible for carcinogen activation.
- A stimulation of intracellular antioxidant defense mechanisms.

These effects result in an inhibition of DNA damage induced by procarcinogens such as AFB_1, B[*a*]P, PhIP and DMBA.

The molecular mechanism of the C + K-mediated protein induction appears to involve the Nrf2 transcription factor through a regulation of ARE-driven gene expression. Such a mechanism is common to many cancer chemopreventive blocking agents.

Available information indicates that the C + K effects observed in animal models may be relevant for the human situation. Altogether, the data on the biological effects of C + K provide a plausible hypothesis to explain some anticarcinogenic effects of coffee observed in human epidemiological studies and in animal experiments.

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