Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food and Chemical Toxicology 43 (2005) 433–441
www.elsevier.com/locate/foodchemtox

Coffee diterpenes prevent the genotoxic effects of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and *N*-nitrosodimethylamine in a human derived liver cell line (HepG2)

B.J. Majer [a,1], E. Hofer [a,1], C. Cavin [b], E. Lhoste [c], M. Uhl [d], H.R. Glatt [e], W. Meinl [e], S. Knasmüller [a,*]

[a] *Institute of Cancer Research, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria*
[b] *Nestlé Research Center, P.O. Box 44, CH-1000 Lausanne 26, Switzerland*
[c] *Institut de la Recherche Agronomique, Domaine de Vilvert, F-78352 Jouy-en-Josas, France*
[d] *Umweltbundesamt, Spittelauer Lände 5, A-1090 Vienna, Austria*
[e] *Department of Toxicology, German Institute of Human Nutrition, Arthur-Scheunert-Allee 114-116, D-14558 Nuthetal, Germany*

Received 28 May 2004; accepted 3 November 2004

Abstract

Aim of the present experiments was to study the genotoxic effects of coffee diterpenoids, namely cafestol palmitate and a mix of cafestol and kahweol (C + K) in human derived hepatoma (HepG2) cells. Furthermore, we investigated the potential protective properties of these substances towards carcinogens contained in the human diet, namely *N*-nitrosodimethylamine (NDMA) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). C + K and cafestol palmitate were tested over a broad dose range in micronucleus (MN) assays and no indication for genotoxic effects was seen. In combination experiments with PhIP (300 μM), pronounced inhibition (≈1.7-fold) of MN formation was observed with C + K and cafestol palmitate at dose levels ⩾0.9 and 1.7 μg/ml, respectively. Enzyme measurements indicate that the protection is due to inhibition of sulfotransferase, an enzyme involved in the activation of the amine, and/or to induction of UDP-glucuronosyltransferase which detoxifies the DNA-reactive metabolites of PhIP. Furthermore, a significant increase of glutathione-*S*-transferase was seen, whereas the activities of cytochrome P-450 1A1 and *N*-acetyltransferase 1 were not significantly altered. Also in combination experiments with C + K and NDMA, strong protective effects (50% reduction of genotoxicity) were seen at low dose levels (⩾0.3 μg/ml). Since inhibition of MN was also observed when C + K were added after incubation with NDMA, it is likely that the chemoprotective effects are due to induction of DNA repair enzymes. Comparison of data on the effects of C + K on the cholesterol metabolism, which was investigated in earlier in vivo studies, with the present findings suggests that DNA-protective effects take place at exposure levels which are substantially lower than those which cause hypercholesterolemia.

Keywords: Coffee diterpenes; Cafestol; Kahweol; *N*-nitrosodimethylamine; PhIP; Metabolic enzymes; Antimutagenicity; Micronucleus; HepG2

Abbreviations: AFB_1, aflatoxin B_1; B(*a*)P, benzo[*a*]pyrene; C + K, cafestol and kahweol; CDNB, 1-chloro-2,4-dinitrobenzene; CYP, cytochrome P-450; DMEM, Dulbecco's minimal essential medium; DMSO, dimethyl sulfoxide; EROD, ethoxyresorufin *O*-deethylase; GST, glutathione-*S*-transferase; MGMT, O^6-methylguanine-DNA methyltransferase; MN, micronuclei; MROD, methoxyresorufin *O*-deethylase; NAT, *N*-acetyltransferase; NDMA, *N*-nitrosodimethylamine; PBS, phosphate buffered saline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; SULT, sulfotransferase; UGT, uridinediphosphate-glucuronosyltransferase

* Corresponding author. Tel.: +43 1 4277 65142; fax: +43 1 4277 9651.
E-mail address: siegfried.knasmueller@meduniwien.ac.at (S. Knasmüller).
[1] The first two authors contributed equally.

0278-6915/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2004.11.009

1. Introduction

A number of epidemiological studies indicate that coffee consumption is inversely related to the incidence of colon cancer in humans (Giovannucci, 1998); additionally, protection towards other forms of cancer has been suggested (Nishi et al., 1996; Inoue et al., 1998) and a number of animal studies support the assumption that coffee is protective against the action of dietary carcinogens which are relevant for humans, such as nitrosamines (Nishikawa et al., 1986) and polycyclic aromatic hydrocarbons (Wattenberg, 1983).

Already more than 20 years ago, results from animal experiments showed that the protection against 7,12-dimethylbenzanthracene-induced tumour formation is caused by a lipid fraction, whose major constituents are the diterpenoids cafestol and kahweol (C + K) (Lam et al., 1982; Wattenberg et al., 1986). The C + K concentrations in coffee depend strongly on the preparation procedure. They are high in unfiltered (Scandinavian and Turkish style) coffee (90–182 mg/l), whereas in paper filtered coffee much lower values (0.2–0.3 mg/l) were found (Gross et al., 1997). These concentrations correspond to an uptake of 45–91 mg/pers/d for unfiltered and 0.1–0.15 mg/pers/d for filtered coffee, based on a consumption of 500 ml/pers/d. The effects of these bioactive compounds were later investigated in a number of chemoprevention studies both in vivo and in vitro and were found to be protective against DNA-reactive carcinogens such as aflatoxin B_1 (AFB_1) (Cavin et al., 1998, 2001), 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP), a heterocyclic amine formed during the cooking of meat (Huber et al., 1997), and benzo[*a*]pyrene (B(*a*)P) (Cavin et al., 2003). Only a few results on protective effects in human cells are available; e.g., Cavin et al. (2001) demonstrated protection against AFB_1 in a human epithelial liver cell line and inhibition of B(*a*)P-DNA binding was demonstrated in a human derived bronchial epithelial cell line (Cavin et al., 2003). In all these experiments, prevention of DNA adduct formation was monitored as an indication of DNA-protective activity but no studies on protection at the chromosomal level were conducted.

Several attempts have been made to elucidate the mechanisms responsible for the antigenotoxic properties of C + K. It was shown that the protection towards AFB_1 is probably due to inhibition of activating enzymes (i.e., CYP3A2 and CYP2C11) and to induction of glutathione-*S*-transferase (GST) (Cavin et al., 2001; Huber et al., 2002). The induction of the latter enzyme plays also a key role in the protective effects towards polycyclic aromatic hydrocarbons (Cavin et al., 2002, 2003). C + K also induce antioxidant enzymes (for review see Cavin et al., 2002) and very recently Huber et al. (2003) reported on the induction of O^6-methylguanine-DNA methyltransferase, a repair enzyme which removes pre-carcinogenic DNA-damage caused by alkylating agents (Kaina et al., 1998).

The present investigation concerns the question if the two coffee diterpenoids are protective towards DNA damage on the chromosomal level in human derived liver (HepG2) cells, which have retained the activities of xenobiotic drug metabolising enzymes in an inducible form (Knasmüller et al., 1998; Majer et al., 2004). These cells proved to be useful for the investigation of dietary antimutagens (Knasmüller et al., 1998; Laky et al., 2002; Kassie et al., 2003a; Mersch-Sundermann et al., 2004) and enable the detection of protective mechanisms, which are not represented in other in vitro models (Knasmüller et al., 2002; Uhl et al., 2003). As an endpoint, we investigated the induction of micronuclei (MN), which are formed as a consequence of chromosome breakage and aneuploidy (Heddle et al., 1984).

In a first experimental series, we tested the effects of cafestol palmitate, and a mix of cafestol and kahweol (C + K), respectively, since it has been shown that many DNA-protective compounds are genotoxic per se at elevated dose levels (Sanyal et al., 1997; von Borstel and Higgins, 1998; Knasmüller et al., 2002; Uhl et al., 2003). In subsequent combination experiments, the protective effect of the coffee diterpenoids towards *N*-nitrosodimethylamine (NDMA) and PhIP was investigated. Both compounds are contained in the human diet and are considered to be involved in the aetiology of cancer in humans (IARC, 1978, 1993; Knekt et al., 1999). In additional experiments, we monitored the effects of C + K on the activities of several drug metabolising enzymes in HepG2 cells, which are involved in the activation and detoxification of these carcinogens, namely cytochrome P-450 (CYP) 1A1, the sulfotransferases (SULT) 1A1 and 1A3, *N*-acetyltransferase (NAT) 1, UDP-glucuronosyltransferase (UGT) and glutathione-*S*-transferase (GST).

2. Material and methods

2.1. Chemicals

Cafestol palmitate and a mixture of cafestol and kahweol (C + K) were prepared from coffee oil according to the procedure of Bertholet (1987). The mixture contained C + K in the proportions 52.5:47.5 and its purity was greater than 98%. 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) was purchased from Toronto Research Chemicals (Toronto, Canada); *N*-nitrosodimethylamine (NDMA) was from Sigma–Aldrich (St. Louis, USA); C + K, cafestol palmitate and PhIP were dissolved in DMSO, NDMA was diluted with PBS. The inorganic salts for buffer solutions and dimethylsulfoxide (DMSO) came from Merck (Darmstadt, Germany); acetonitrile, acetyl phosphate, acetyl-coenzyme