Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 757–763
Journal of Nutritional Biochemistry

The role of epoxidation and electrophile-responsive element-regulated gene transcription in the potentially beneficial and harmful effects of the coffee components cafestol and kahweol ☆

Saskia T.J. van Cruchten[a,b,*], Laura H.J. de Haan[c], Patrick P.J. Mulder[d], Cindy Kunne[e], Mark V. Boekschoten[a,b], Martijn B. Katan[f], Jac M.M.J.G. Aarts[c], Renger F. Witkamp[a]

[a]Department of Human Nutrition, Wageningen University, 6700 EV, Wageningen, The Netherlands
[b]Top Institute of Food and Nutrition, 6709 PA, Wageningen, The Netherlands
[c]Department of Toxicology, Wageningen University, Wageningen, The Netherlands
[d]RIKILT, - Institute of Food Safety, 6700 EA, Wageningen, The Netherlands
[e]Liver Center, Academic Medical Center, 1105 BK, Amsterdam, The Netherlands
[f]Institute of Health Sciences, VU University Amsterdam, 1081 HV, Amsterdam, The Netherlands

Received 19 January 2009; received in revised form 4 May 2009; accepted 8 May 2009

Abstract

Cafestol and kahweol are diterpene compounds present in unfiltered coffees. Cafestol is known as the most potent cholesterol-raising agent that may be present in the human diet. Remarkably, the mechanisms behind this effect have only been partly resolved so far. Even less is known about the metabolic fate of cafestol and kahweol. From the structure of cafestol, carrying a furan moiety, we hypothesized that epoxidation may not only be an important biotransformation route but that this also plays a role in its effects found. In bile duct-cannulated mice, dosed with cafestol, we were able to demonstrate the presence of epoxy-glutathione (GSH) conjugates, GSH conjugates and glucuronide conjugates. In addition, it was shown that cafestol was able to induce an electrophile-responsive element (EpRE). Using a murine hepatoma cell line with a luciferase reporter gene under control of an EpRE from the human NQO1 regulatory region, we also found that metabolic activation by CYP450 enzymes is needed for EpRE induction. Furthermore, raising intracellular GSH resulted in a decrease in EpRE-mediated gene induction, whereas lowering intracellular GSH levels increased EpRE-mediated gene induction. In conclusion, evidence suggests that cafestol induces EpRE, apparently via a bioactivation process that possibly involves epoxidation of the furan ring. The epoxides themselves appear subject to conjugation with GSH. The effects on EpRE can also explain the induction of GSH which seems to be involved in the reported beneficial effects of cafestol, for example, when administered with aflatoxin B1 or other toxic or carcinogenic compounds.

Keywords: Cafestol; Epoxidation; Diterpenes; Metabolism; Electrophile-responsive element; Glutathione

1. Introduction

Cafestol and kahweol (Fig. 1) are diterpenes mainly present in unfiltered coffees such as French press, espresso and boiled coffees [1–4]. Depending on the quality/blend and process of coffee preparation [5], concentrations of cafestol and its structural analogue kahweol are each estimated to range between 0.1 and 7 mg/ml coffee [2]. Cafestol is now regarded as the most potent cholesterol-elevating compound known in the human diet [1,4,6] and most likely responsible for the association between consumption of boiled coffee and an elevated risk for cardiovascular disease [4,7–9]. Although several potential targets involved in cholesterol homeostasis have previously been proposed, many questions regarding its mechanism have remained unsolved [1,2,8,10]. In addition, there is still very little information on the metabolic fate of the compound in the body. In contrast to its potential harmful effects, cafestol has also been suggested to have anticarcinogenic or hepatoprotective effects [11–17]. In rodent studies, the compound has been shown to decrease the activity of several CYP450 enzymes and to induce glutathione-S-transferase (GSTs) [13,15,18–20]. In humans, consumption of unfiltered coffee or cafestol leads to elevation of glutathione (GSH) content in the colorectal mucosa and in plasma [21]. In rats treated with a 1:1 mixture of cafestol and kahweol (C/K), a dose-dependent increase of GSH is seen in the liver and colon. In these rats an increase in glutamylcysteine synthetase, the first and rate-limiting enzyme in the GSH biosynthesis pathway, is also seen [22,23].

☆ This work was supported by a grant of the TI Food & Nutrition.
* Corresponding author. Division of Human Nutrition, 6703 HD Wageningen, The Netherlands. Tel.: +31 0 317 480403; fax: +31 0 317 483342.
E-mail address: Saskia.vancruchten@wur.nl (S.T.J. van Cruchten).

0955-2863/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2009.05.001

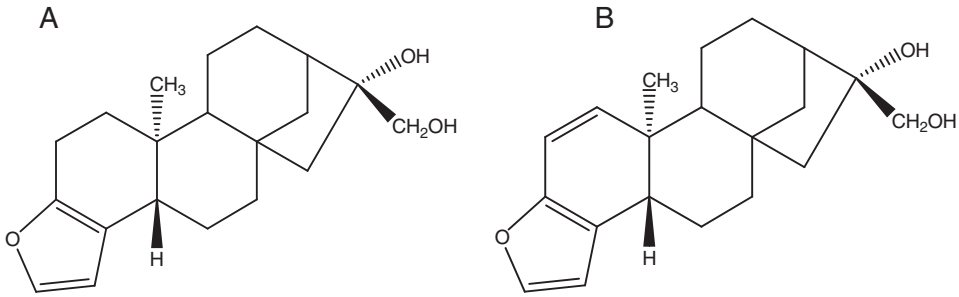

Fig. 1. Structural formulas of cafestol (A) and kahweol (B).

Finally, cafestol and/or kahweol and coffee brews that contain these diterpenes cause elevations of serum alanine aminotransferase (ALAT) and to a lesser extent aspartate aminotransferase (ASAT) and a temporary depression of gamma glutamyl transferase in plasma [4,10,24].

From the structure of cafestol, which contains a furan moiety, we hypothesized that this apparently two-faced behavior might be related to a bioactivation which includes the formation of one or more furan epoxides. A similar reaction has been described for example for the natural toxin ipomeanol [25]. On the one hand, such furan epoxides could be involved in the hepatotoxic and cholesterol-elevating effects. On the other hand, epoxidations could explain the induction of GSH biosynthesis, GST and other chemo protective enzyme genes, possibly mediated by an electrophile-responsive element (EpRE) in the regulatory region of the genes involved. The EpRE is a regulatory sequence mediating the coordinated transcriptional activation of genes associated with Phase 2 biotransformation, protection against oxidative stress and other cancer-chemo protective mechanisms [26]. The key regulator of EpRE-mediated gene expression is the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and, to a lesser extent, Nrf1 [27]. Very recently, it was shown that coffee may protect against the adverse effects of aflatoxin B1 via stimulation of the Nrf2-antioxidant-responsive element (ARE) pathway [12].

The aim of the present study was to further investigate this potential mechanism and the role of bioactivation via epoxide formation for the specific coffee components cafestol and kahweol. First, a study was undertaken to identify GSH conjugates of cafestol with liquid chromatography-mass spectrometry (LCMS) in bile-duct-cannulated mice. After this, a series of experiments were carried out using a hepa-1c1c7 cell line stably transfected with a luciferase reporter gene under control of an EpRE from the human NQO1 regulatory region to obtain a reporter cell line responsive to electrophiles. The induction of EpRE-mediated gene expression was studied after exposure to cafestol and kahweol with and without preincubation with S9-mix, a preparation of enzymes of xenobiotic metabolism, particular of phase 1. Therefore, the experiments allowed a determination of the role of metabolic activation in case of cafestol. Furthermore, to investigate whether oxidation was the mechanism leading to Nrf2 release and subsequent gene induction, we modulated the intracellular GSH level using N-acetyl-L-cysteine (NAC) and buthionine-sulfoximine (BSO) to respectively increase and decrease the intracellular GSH concentration.

2. Methods

2.1. Chemicals

Alpha-Modified Eagle's medium (α-MEM), Hanks balanced salt solution, Trypsin, foetal calf serum (FCS), phosphate-buffered saline (PBS), gentamicin and G418 were purchased from Gibco Invitrogen Corporation (Breda, The Netherlands). Dimethyl sulfoxide (DMSO) was obtained from Acros Organics (Morris Plains, NJ, USA). P450 inhibitor, 1-aminobenzotriazole (ABT), was obtained from Sigma Chemical (St. Louis, MO, USA) as were potassium phosphate, $NADP^+$, glucose-6-phosphate, MgCl2 and glucose-6-phosphate dehydrogenase. Clinoleic (20%) was obtained from Baxter (Deerfield, IL, USA). Cafestol was obtained from LKT Laboratories (St. Paul, MN, USA) and the mixture kahweol/cafestol [K 30.1%/C 61.9%] was isolated from coffee oil by our department [7]. Kahweol as single compound could not be obtained in sufficient quantities.

For LC-MS analysis, we used water LC-MS grade and methanol high-performance liquid chromatography (HPLC) supra gradient grade purchased from Biosolve (Valkenswaard, The Netherlands). Formic acid, was obtained from Merck (Darmstadt, Germany). Ammonium formate 97% was obtained from Sigma-Aldrich (St. Louis).

2.2. Cell line

Hepa-1c1c7 mouse liver hepatoma cells were a kind gift from Dr. M.S. Denison, (University of California, Davis) and were stably transfected with the reporter vector pTI(hNQO1-EpRE)Luc+ carrying the EpRE from the human NQO1 gene regulatory region between basepair −470 and −448 from the transcription initiation site (5′-AGT CAC AGT GAC TCA GCA GAA TC-3′) coupled to a luciferase reporter gene (LUX), resulting in the EpRE(hNQO1)-LUX cell line as described elsewhere [28], and referred to as EpRE-LUX in this article. EpRE cells were cultured in α-MEM, supplemented with 10% FCS and 50 μg/ml gentamicin and in addition 0.5 mg/ml G418 to maintain selection pressure on the presence of the reporter gene insertion. The cells were maintained in a humidified atmosphere with 5% CO_2 at 37°C.

2.3. Animals

Pure-bred, wild-type adult, male (C57BL6/j) mice were purchased from Harlan (Horst, The Netherlands). Mice were housed in a light- and temperature-controlled facility and had free access to water and standard laboratory chow (RMH-B, Hope farms, Woerden, The Netherlands). All animal studies were approved by the local committee for care and use of laboratory animals.

2.4. Animal experiment

Five C57BL6/j mice were fasted overnight. Gall bladder cannulation and bile collection was performed as described previously [29]. To maintain a constant bile flow of 300 μl/h per 25 g body weight (BW), mice were infused in the jugular vein with a bile acid solution containing 30 mM tauroursodeoxycholic acid according to standard procedures, also with a flow rate of 300 L/h per 25 gram BW) [29]. Sixty minutes after infusion, bile flow was considered stable, and cafestol was injected through the portal vein. Subsequently, cafestol (total dose 12 μg) dissolved in 20% Clinoleic was administrated through the portal vein. Bile was sampled every 15 min for the first hour and every 30 min after the first hour. In total, bile was sampled for 5 h. Bile samples were immediately frozen at −80°C and subsequently analysed by LC-MS. Bile flow was determined gravimetrically assuming a density of 1 g/ml for bile.

2.5. LCMS analyses

All LC-MS analyses were carried out using a Waters Acquity ultra performance liquid chromatography system coupled to a Waters LCT Premier time-of-flight mass spectrometer (ToFMS). Chromatographic separation was conducted using a Zorbax Eclipse XDB C8 (Agilent) column (150×2.1 mm injected dose, 5 μm) thermostatted at 30°C. The injection volume was 5 μl. For analyses carried out in the positive ion electrospray ionization (ESI+) mode, solvent A consisted of 0.02% formic acid in water and solvent B of 0.02% formic acid in methanol. Gradient elution was linearly programmed as follows: 0 min 5% B, 1 min 5% B, 19 min 95% B and 21 min 5% B at a flow rate of 0.2 ml/min. For negative ion ESI (ESI−) analyses solvent A consisted of 5 mM ammoniumformate in water, and solvent B was methanol. The following linear gradient was used: 0 min 5% B, 1 min 5% B, 16 min 80% B and 18 min 5% B at a flow rate of 0.2 ml/min.

The ToFMS was equipped with a LockSpray dual ESI source. Source operating conditions were 120°C ion source temperature, 350°C desolvation temperature, 500 L/h desolvation gas flow rate and 50 L/h cone gas flow rate. The spray voltage was maintained at 3 kV for ESI+ and −2.5 kV for ESI−. Mass spectra were acquired in centroid mode with internal mass correction by scanning from 100 to 1000 m/z. The lock masses used for ToF mass correction in ESI+ and ESI− mode were the $[M+H]^+$ ion (attenuated lock mass) and

^{13}C isotope $[M+H]^+$ ion or the $[M-H]^-$ ion (attenuated lock mass) and ^{13}C isotope $[M-H]^-$ ion of leucine enkephalin, respectively. Leucine enkephalin (1 ng/μl in water/acetonitrile 80:20 v/v) was delivered to the ion source at 10 μl/min using an HPLC pump. The analyte to reference scan ratio was 9:1. Experiments were performed at spectral acquisition time of 1.0 s. The resolution of the ToFMS was ~10 000 full width at half maximum (FWHM). Dynamic range enhancement was switched on.

2.6. Metabolic activation of cafestol

To study the role of metabolic activation, we added S9 mix containing 10% S9 enzyme fraction (purchased from Trinova Biochem, Giessen, Germany), which was prepared from the liver of male Sprague–Dawley rats treated with Aroclor-1254. S9 mix contained an NADPH-generating system consisting of 1.3 M nicotinamide adenine dinucleotide phosphate (NADP+), 3.3 M glucose-6-phosphate and 0.4 U/ml glucose-6-phosphate dehydrogenase dissolved in 0.5 M phosphate buffer, 0.08 M $MgCl_2$ and 0.330 M KCl prepared with milliQ water. The S9 mix was used at a concentration of 10% in experimental medium. Medium with or without S9 mix and with cafestol or a mixture of cafestol/kahweol was preincubated for 1 h at 37°C in a rotating bath at 300 rpm. After preincubation, the mixture was added to the cells, which were exposed for 6 h, where after the cells were harvested and analyzed. GSH levels in the cells were modified by treatment with either *N*-acetyl-L-cysteine (NAC, 40 mM) to generate higher levels of GSH and buthionine-sulfoximine (BSO, 100 μM) to decrease intracellular GSH levels.

Inhibition of certain P450 enzymes was achieved by exposing the cells for 30 min with 1-ABT (50 μM) [30,31] and NADPH generating system. ABT inhibits specific P450 enzymes such as CYP3A4, CYP2E1, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP1A2 [30,31]. However, it predominantly inhibits CYP3A4. Then cells were incubated with α-MEM, S9 mix, cafestol and 50 μM 1-ABT.

2.7. EpRE-LUX

EpRE-mediated induction of gene expression by cafestol and a mixture of cafestol/kahweol was tested using the EpRE-LUX luciferase reporter gene assay as described previously [28,32]. Briefly, EpRE-LUX cells were propagated as described above plated in culture medium in 96-wells view-plates (PerkinElmer, 2×10^4 cells/100 μl/well) and incubated for 24 h to allow attachment of the cells to the bottom of the wells and the formation of a confluent monolayer. Next, the culture medium was removed and cells were exposed to 100-μl medium (FCS-free) containing different concentrations of cafestol and S9 mix. S9 mix was added to ensure metabolisation of the test compounds with a full spectrum of active P450 enzymes (S9 mix contained an NADPH-generating system consisting of 1.3 M nicotinamide adenine dinucleotide phosphate (NADP+), 3.3 M glucose-6-phosphate and 0.4 U/ml glucose-6-phosphate dehydrogenase dissolved in 0.5 M phosphate buffer, 0.08 M $MgCl^2$ and 0.330 M KCl prepared with milliQ water). Medium with different cafestol concentrations and S9 mix were preincubated for 1 h to allow metabolite formation. DMSO concentration in the culture medium was kept constant at 0.4%. After different periods of exposure, cells were washed with 0.5×concentrated PBS and lysed by addition of low salt buffer [10 mM Tris, 2 mM dithiothreitol (DTT) and 2 mM *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetra-acetic acid monohydrate; pH 7.8] followed by one freezing and thawing cycle. Luciferase reagent [20 mM Tricine, 1.07 mM $(MgCO_3)_4Mg(OH)_2$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 2 mM DTT, 0.47 mM D-luciferin, 5 mM adenosine-5′-triphosphate (ATP); pH 7.8] was injected and luciferase activity was immediately measured using a Luminoskan RS (Labsystems) luminometer. The luciferase expression measured was expressed as the induction factor (IF) defined as the potency of cafestol to increase the luciferase expression as compared to cells incubated with medium containing only 0.4% DMSO.

2.8. Statistical analysis

Comparisons between a single control and treatment means were made by using post hoc tests: Bonferroni and a Dunnett's test. Comparisons between metabolically activated and metabolically unactivated cells were made by paired Student's *t* test. The limit of statistical significance was set at $P<.05$. Statistical analysis was performed using SPSS 15.

3. Results

3.1. Identification of cafestol metabolites in bile

Three mice were injected with cafestol and two mice with vehicle as a control. Bile fluid was sampled and analysed for cafestol metabolites by LC-ToF-MS. Only peaks that were detected in the bile fluid of all three treated mice and were absent in the control mice were considered potential metabolites of cafestol. Fig. 2 shows the extracted ion chromatogram (EIC) recorded in ESI+ of a candidate GSH metabolite of cafestol. In the chromatogram, a de-protonated molecular ion ($[M+H]^+$) is present of a compound with a retention time of 10.06 min (Fig. 2A). This compound was not present in the EIC of the mice that had not received cafestol (Fig. 2B). The mass spectrum acquired of the compound at 10.06 min is shown in Fig. 3; for the $[M+H]^+$ ion, a mass is observed of 656.2855 Da. This matches closely with the elemental composition $C_{30}H_{46}N_3O_{11}S$, for which a theoretical mass of 656.2848 Da is calculated. The difference between the measured and theoretical mass of the $[M+H]^+$ ion is 1.1 ppm (0.7 mDa). Similarly, when the same sample was recorded in ESI−, a deprotonated molecular ion ($[M-H]^-$) was found at a retention time of 5.66 min (please note that a different mobile phase composition and gradient was used), with an observed mass of 654.2682 Da (data not shown). The observed mass matches with an elemental composition $C_{30}H_{44}N_3O_{11}S$ with a deviation of −3.1 ppm (−2.0 mDa).

Taking into account the mass of cafestol epoxide (332.1988 Da) and that of GSH (307.0838 Da), the expected adduct, formed by conjugation

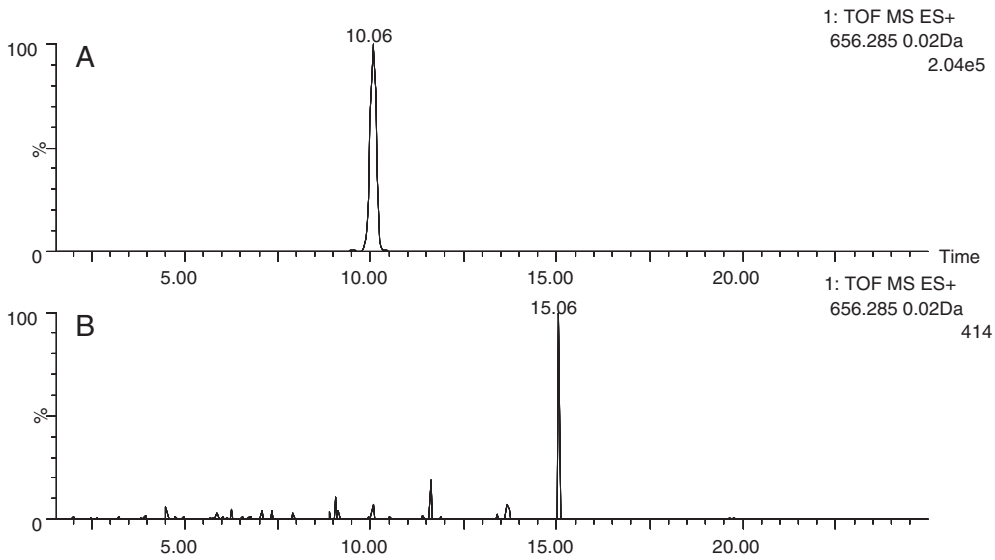

Fig. 2. Detection of a potential GSH metabolite of cafestol by using LC-ToFMS in ESI+ mode. EIC for mass 656.285 Da using a mass tolerance window of 10 mDa and normalized to the highest peak. (A) EIC obtained for a mouse treated with cafestol. The peak at 10.06 min in the bile fluid of the cafestol treated mouse is absent in the control mouse. (B) EIC obtained for a non-treated (control) mouse.

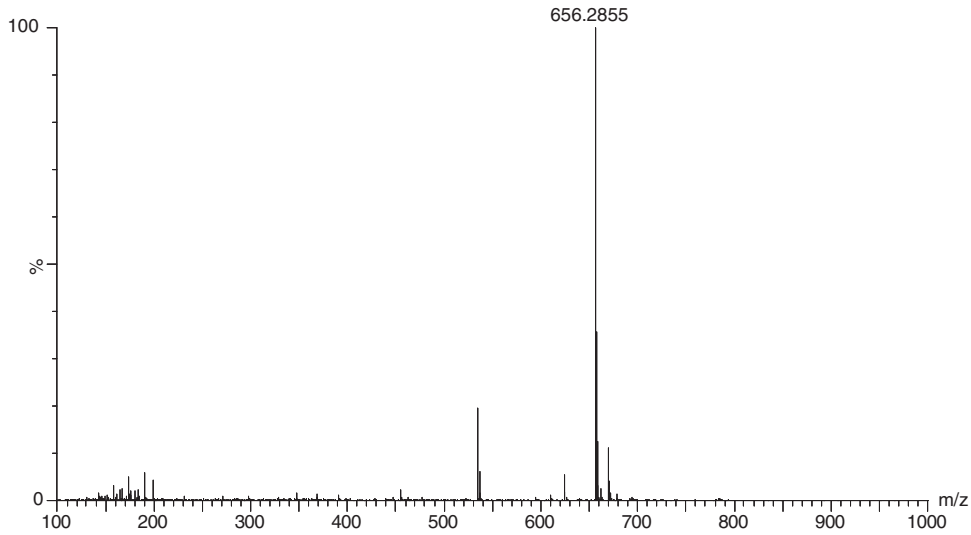

Fig. 3. ESI+ mass spectrum obtained for the component at a retention time of 10.06 min present in the bile of a mouse treated with cafestol. The $[M+H]^+$ ion with a measured mass of 656.2855 is tentatively identified as a GSH conjugate of 2-hydroxy-cafestol epoxide.

of GSH with cafestol epoxide, will have a molecular mass of 640.2898 Da and $C_{30}H_{46}N_3O_{10}S$ as elemental composition when measured as the $[M+H]^+$ ion and a mass of 638.2753 Da and $C_{30}H_{44}N_3O_{10}S$ as elemental composition when measured as the $[M-H]^-$ ion. The difference between the observed metabolite and the expected cafestol epoxide GSH adduct equals one oxygen atom. Apparently, the major GSH adduct present in bile is an oxygenated metabolite.

At this point, it remains rather speculative at which position of the molecule oxidation has occurred and at which stage during metabolism. Possible sites of oxidation are the remaining furan double bond (formation of an epoxide), oxidation of the GSH sulfur (formation of sulfoxide) and hydroxylation at the methylene position next to the furan ring. In the latter case, hydroxylation may take place before epoxidation producing 2-hydroxy cafestol. Subsequent epoxidation to 2-hydroxy cafestol epoxide and conjugation with GSH will produce the putative metabolite (Fig. 4). Hydroxylation of steroids is a common metabolism route and may take place to increase the polarity of the lipophilic cafestol. Without the collection of tandem mass spectrometry (MSMS) fragmentation spectra, and preferably, the isolation of the metabolite and subsequent nuclear magnetic

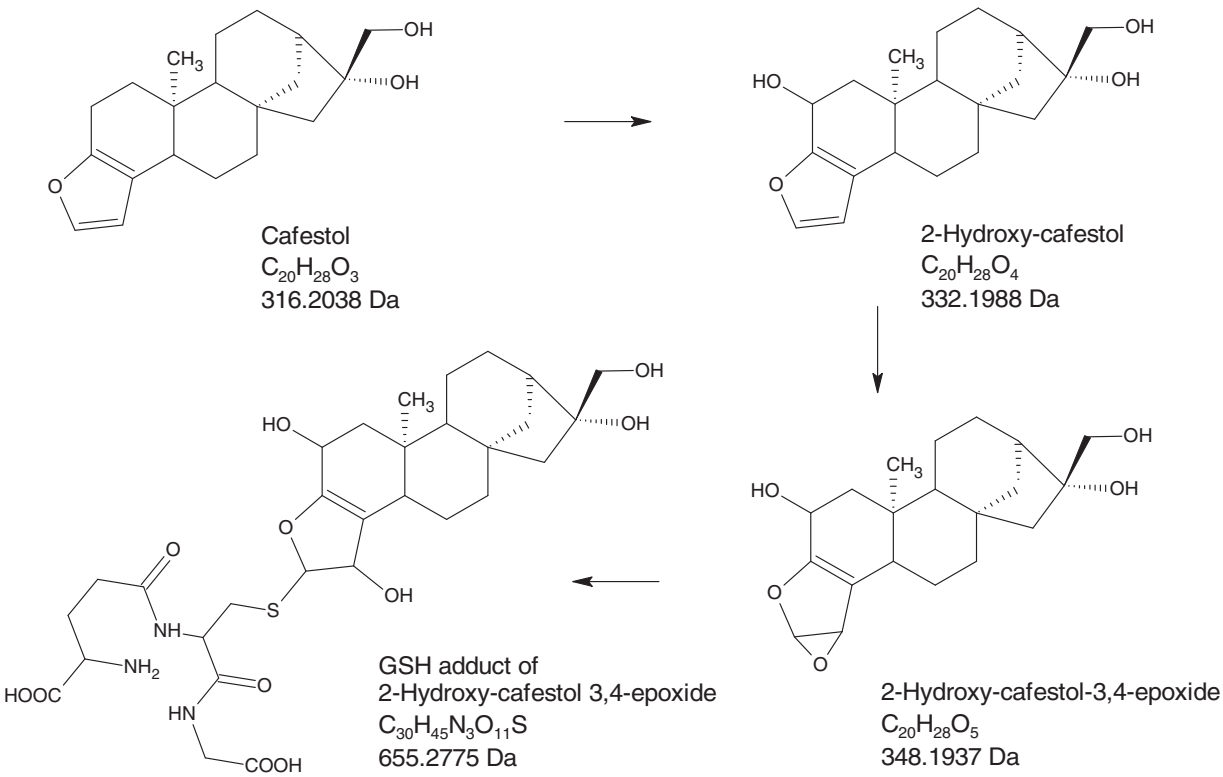

Fig. 4. Proposed schematic representation of the formation of a GSH conjugate of cafestol. In this scheme cafestol is hydroxylated prior to epoxidation of the furan moiety of cafestol by P450 enzymes.

resonance analysis, the exact structure of this metabolite can not be determined with certainty. The collected amounts of bile fluid were too small to allow such a detailed analysis, however.

At least two other GSH containing metabolites were detected in the bile fluids of cafestol treated mice (data not shown). Considering the mass of these metabolites, they contain an extra oxygen compared to the 655 Da metabolite.

Next to GSH, conjugates of cafestol, also a glucuronide conjugate, were detected in the bile.

3.2. EpRE-mediated effects of cafestol and kahweol

In the EpRE-Lux cells exposed to cafestol, an obvious luciferase induction response (IF) is only seen after metabolic stimulation (Fig. 5, $P<.001$). A mixture of cafestol/kahweol (68.2% cafestol, 31.8% kahweol) induced EpRE-mediated gene induction at a concentration of 50 μM (34.1 μM cafestol+15.9 μM kahweol) after 6 h of exposure (Fig. 6). However, at high concentrations of the mixture, C/K (200 and 300 μM, total amount diterpenes are similar in treatments) cells died, whereas similar cafestol concentrations showed no effect on cell viability (morphologically checked). Increase of intracellular GSH levels by NAC resulted in a 4.5- to 5-fold decrease of EpRE-mediated gene induction as compared to control without NAC treatment (Fig. 7). When cells were GSH depleted by means of pretreatment with BSO, metabolically activated cafestol caused a 2.5-fold increase of EpRE-mediated gene induction compared to exposed cells with unchanged GSH levels (Fig. 7). Inhibition of specifically P450 enzymes, by adding ABT [30] resulted in a lower induction of the EpRE-controlled reporter gene expression compared to control (data not shown). ABT inhibits specific p450 enzymes such as CYP3A4, CYP2E1, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP1A2 [30,31]. Therefore, we conclude that P450 enzymes such as CYP3A4 are essential for metabolisation and epoxidation of cafestol.

4. Discussion

It has become clear from several studies that the effects of cafestol on human health can be positive and negative. The beneficial effects include a possible reduction of toxicant activity after consumption of cafestol, whereas the increase in plasma cholesterol concentration can be considered as potentially harmful. Both outcomes are considered as relevant for a normal coffee consumption pattern [1,2,4,7,8,33–36]. The present study contributes to a further mechanistic understanding of this balance between beneficial and potentially detrimental effects of cafestol.

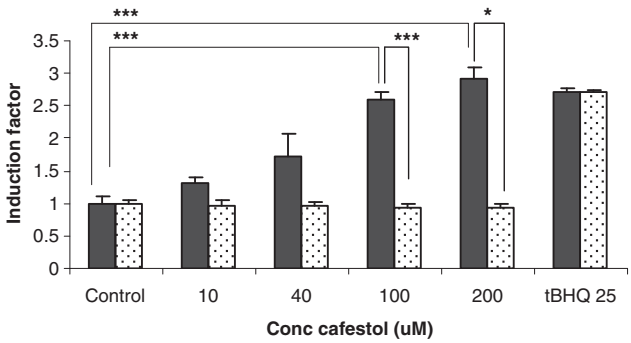

Fig. 5. Induction of EpRE-mediated gene transcription in a murine hepatoma cell line by cafestol . (■ with metabolic activation ▒ without metabolic activation). TBHQ (25 μM) is used as a positive control for EpRE induction. Data are presented as means with standard deviation based on six independent measurements. Treatments groups 100 and 200 μM cafestol differ significantly compared to control ($P\leq.001$). Metabolic activated cells (■) differ significantly from control cells (▒).***$P\leq.001$; *$P\leq.002$.

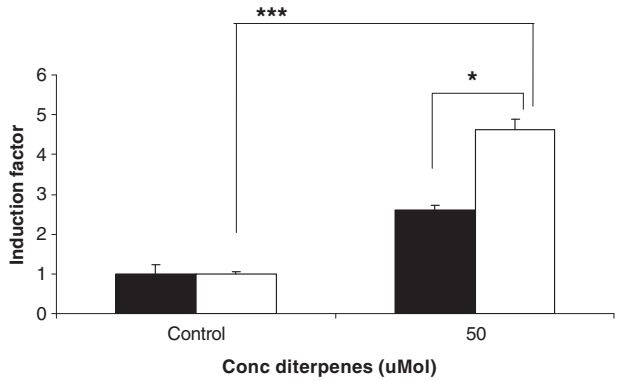

Fig. 6. A mixture of cafestol and kahweol induces more EpRE-mediated gene induction compared to cafestol alone. Murine hepatoma cells treated with a mixture of cafestol/kahweol (□) induce more EpRE-mediated activation compared to cafestol treated cells (■). Data are presented as means with standard deviation based on six independent measurements. ***$P\leq.001$; *$P\leq.05$.

We provide evidence that the metabolism of cafestol plays a key role in the induction of GSTs and other Phase II enzymes via the EpRE. Epoxidation of the furan moiety is probably involved in this activation. EpRE-, initially referred to as ARE is an important gene-regulatory enhancer mediating induction of the expression of a battery of genes involved in the defense against electrophilic and other reactive species. From the structure of cafestol, we predicted that epoxidation in the furan moiety is a possible biotransformation route. A number of metabolites were found in bile of the bile-duct-cannulated mice. Further analysis revealed the identity of other cafestol metabolites including epoxy-GSH conjugates and GSH conjugates. Epoxidation has been described for several other furan containing molecules, including furan, menthofuran, ipomeanine, 4-ipomeanol, furosemide and teucrin A [25,37–40]. For some of these compounds, including the natural toxin ipomeanol, the role of specific CYP450s has been established and furan-epoxides have been associated with cellular toxicity [25]. To the best of our knowledge, the formation of epoxides has not been described before for cafestol.

An important role in the potential anticarcinogenic properties of cafestol has been attributed to both a decreased activity of bio-activating CYP450 enzymes or to an induction of GSTs. Evidence for a decrease of CYP450 expression or activity by cafestol is coming from a number of studies. For example, Huber et al. [13] recently demonstrated inhibition of a fairly wide pattern of P450 enzymes in rats that had received a cafestol/kahweol mixture. The effect was most

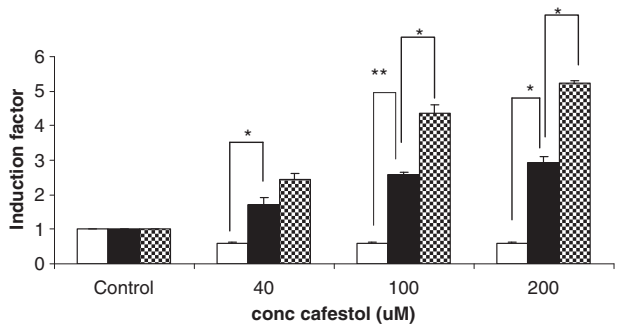

Fig. 7. Effects of changes in intracellular GSH levels on cafestol induced EpRE activity in murine hepatoma cells. □, GSH increase (NAC); ■, with metabolic activation ▒ GSH depletion (BSO). *$P<.001$; **$P=.076$. An increase in total GSH concentration (□ NAC) results in a significant decrease in cafestol induced EpRE induction. In GSH depleted cells (▒ BSO), cafestol induced EpRE induction increases 2.5-fold. Data are presented as means with standard deviation based on six independent measurements.

pronounced with CYP1A2 for which inhibition was detectable at the level of activity and mRNA. Interestingly, this P450 enzyme is generally associated with bioactivation of several procarcinogens, which often includes the formation of an epoxide. Similar conclusions were obtained by Cavin et al. [11] who studied the effect of coffee in rats and human hepatocytes. Also in this study, CYP1A1/2 was inhibited in rats and hepatocytes, as was the rat-specific CYP2C11. By contrast, CYP4503A enzymes were not affected by coffee in this study. However, inhibition of several P450 enzymes including CYP3A4 by ABT in our EpRE experiments showed a significant decrease in EpRE-mediated gene induction.

Evidence for a GST-inducing effect by cafestol and/or kahweol is obtained from a number of studies, including [1,12]. This induction can lead to increased GSH conjugation of potentially toxic or carcinogenic compounds, as has been described for aflatoxin B1 [41], azoxymethane [42] and carbon tetrachloride [16].

In contrast to these potentially beneficial effects, cafestol is currently regarded as the most potent cholesterol-elevating bioactive compound that may be present in the human diet [1]. Remarkably, the precise mechanisms behind this effect are still not completely known. It appears that a number of key enzymes and transporters that are involved in the metabolism of cholesterol, including CYP7A1, sterol 12α-hydroxylase and Na^+-taurocholate cotransporting polypeptide can be down-regulated by cafestol. Many of these enzymes are under control of the farnesoid X- (FXR) and the pregnane X (PXR) receptors [1]. At the same time, cafestol may also have direct toxic effects on the liver. It was shown that coffee oil and cafestol (and also kahweol) can cause an increase of serum ALAT and to a lesser extent ASAT in a majority of the subjects [43].

There are some studies that indicate that PXR and FXR also regulate GSTs [1,44,45]. However, recent evidence suggests that the induction of GSTs by cafestol is due to an induction of the Nrf2-mediated gene expression pathway [12,46]. Nrf2 is the key regulator of EpRE-mediated gene expression. It belongs to the nuclear basic leucine zipper transcription factors. The major regulator of Nrf2 is supposed to be Keap1, which represses Nrf2 transcription activation by cytoplasmic sequestration and mediation of the degradation of Nrf2 [27,47,48]. When the disulfide bridges of Keap1 are oxidised, Nrf2 is released from Keap1 [27].

In the present study, we used a stably transfected Hepa1c1c7 cell line with a luciferase reporter gene under the control of the EpRE derived from the human NQO1 gene as previously described [28]. Cafestol caused a dose-dependent luciferase induction. However, this induction was only seen in the presence of S9 mix, suggesting the need for a metabolic activation process. When combined with P450 inhibitors such as ABT [30,31] specifically inhibiting CYP3A4, CYP2B6, CYP2E1, CYP2C9, CYP2C19, 2D6 and CYP1A2, the EpRE induction was significantly decreased. This finding supports the possible role of different P450 enzymes such as CYP3A4 and CYP2E1 in cafestol metabolism. Furthermore, these findings also suggest a role of CYP450 in the bioactivation which possibly occurs via epoxidation. Depletion of GSH (by BSO) was found to cause an increase of EpRE-mediated gene induction, whereas elevation of intracellular GSH (by NAC) inhibited EpRE-mediated gene induction. This is in line with our analytical data showing that GSH conjugation is probably an important elimination route for cafestol and/or prevents the formation of epoxide metabolites capable of oxidation reactions with Keap1 thiol groups critical for Nrf2 release and activation of EpRE-controlled gene transcription. Data from the present study suggest that epoxide formation in the furan ring is probably a key step in this process [37]. So far, it is not clear whether epoxide formation also plays a role in the apparently transient liver toxicity of cafestol, as shown by elevated ASAT and ALAT levels. From its structure, it could be predicted that the other diterpene in coffee, kahweol would be an even more potent inducer of the EpRE at least in vitro, since the double bond between Position 1 and 2 makes it more prone to epoxidation. This is in line with our experimental results. Interestingly, there are some indications that certain coffee blends and mixtures with higher kahweol contents produce a higher ALAT/ASAT response in humans. However, further investigation is needed to verify this. It is not clear to what extent epoxidation or GSH conjugation may contribute to the cholesterol-elevating effect of cafestol. One possibility might be that the GSH conjugate after removal of the glutamyl (by gamma-glutamyl transferases) and glycine moieties is metabolized to a (toxic) compound that affects bile production. Interestingly, while the effects of ASAT and ALAT are acute, cholesterol levels slowly increase. This would be consistent with a gradual increase in GSH conjugation due to induction of gene expression as found in the present study.

In conclusion, we provide evidence that cafestol induces EpRE, apparently via a bioactivation process that possibly involves epoxidation of the furan ring. The epoxides themselves appear subject to conjugation with GSH. Further studies should further reveal the role of the epoxide(s) and the GSH conjugates in the elevation of plasma cholesterol caused by the coffee components cafestol and kahweol.

Acknowledgments

We would like to thank Prof M Müller, Prof. R Oude Elferink and Dr G. Hooiveld for their contribution to the cafestol project.

References

[1] Ricketts ML, Boekschoten MV, Kreeft AJ, Hooiveld GJ, Moen CJ, Muller M, et al. The cholesterol-raising factor from coffee beans, cafestol, as an agonist ligand for the farnesoid and pregnane X receptors. Mol Endocrinol 2007.
[2] Ranheim T, Halvorsen B. Coffee consumption and human health — beneficial or detrimental? — Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and Type 2 diabetes mellitus. Mol Nutr Food Res 2005;49(3):274–84.
[3] Halvorsen B, Ranheim T, Nenseter MS, Huggett AC, Drevon CA. Effect of a coffee lipid (cafestol) on cholesterol metabolism in human skin fibroblasts. J Lipid Res 1998;39(4):901–12.
[4] Urgert R, Katan MB. The cholesterol-raising factor from coffee beans. Annu Rev Nutr 1997;17:305–24.
[5] Speer K, Kölling-Speer I. The lipid fraction of the coffee bean. Braz J Plant Physiol 2006;18(1):201–16.
[6] Weusten-Van der Wouw MP, Katan MB, Viani R, Huggett AC, Liardon R, Liardon R, et al. Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes. J Lipid Res 1994;35:721–33.
[7] Urgert R, Schulz AG, Katan MB. Effects of cafestol and kahweol from coffee grounds on serum lipids and serum liver enzymes in humans. Am J Clin Nutr 1995;61(1):149–54.
[8] de Roos B, Katan MB. Possible mechanisms underlying the cholesterol-raising effect of the coffee diterpene cafestol. Curr Opin Lipidol 1999;10(1):41–5.
[9] Urgert R, Essed N, van der Weg G, Kosmeijer-Schuil TG, Katan MB. Separate effects of the coffee diterpenes cafestol and kahweol on serum lipids and liver aminotransferases. Am J Clin Nutr 1997;65(2):519–24.
[10] Boekschoten MV, Hofman MK, Buytenhek R, Schouten EG, Princen HM, Katan MB. Coffee oil consumption increases plasma levels of 7alpha-hydroxy-4-cholesten-3-one in humans. J Nutr 2005;135(4):785–9.
[11] Cavin C, Holzhaeuser D, Scharf G, Constable A, Huber WW, Schilter B. Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity. Food Chem Toxicol 2002;40(8):1155–63.
[12] Higgins LG, Cavin C, Itoh K, Yamamoto M, Hayes JD. Induction of cancer chemopreventive enzymes by coffee is mediated by transcription factor Nrf2. Evidence that the coffee-specific diterpenes cafestol and kahweol confer protection against acrolein. Toxicol Appl Pharmacol 2008;226(3):328–37.
[13] Huber WW, Rossmanith W, Grusch M, Haslinger E, Prustomersky S, Peter-Vorosmarty B, et al. Effects of coffee and its chemopreventive components kahweol and cafestol on cytochrome P450 and sulfotransferase in rat liver. Food Chem Toxicol 2008;46(4):1230–8.
[14] Huber WW, Scharf G, Nagel G, Prustomersky S, Schulte-Hermann R, Kaina B. Coffee and its chemopreventive components kahweol and cafestol increase the activity of O6-methylguanine-DNA methyltransferase in rat liver — comparison with phase II xenobiotic metabolism. Mutat Res 2003;522(1-2):57–68.
[15] Huber WW, Teitel CH, Coles BF, King RS, Wiese FW, Kaderlik KR, et al. Potential chemoprotective effects of the coffee components kahweol and cafestol palmitates via modification of hepatic *N*-acetyltransferase and glutathione S-transferase activities. Environ Mol Mutagen 2004;44(4):265–76.

[16] Lee KJ, Choi JH, Jeong HG. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. Food Chem Toxicol 2007;45(11):2118–25.
[17] Wattenberg LW. Inhibition of neoplasia by minor dietary constituents. Cancer Res 1983;43(5 Suppl):2448s–53s.
[18] Huber WW, Parzefall W. Modification of *N*-acetyltransferases and glutathione S-transferases by coffee components: possible relevance for cancer risk. Methods Enzymol 2005;401:307–41.
[19] Huber WW, Prustomersky S, Delbanco E, Uhl M, Scharf G, Turesky RJ, et al. Enhancement of the chemoprotective enzymes glucuronosyl transferase and glutathione transferase in specific organs of the rat by the coffee components kahweol and cafestol. Arch Toxicol 2002;76(4):209–17.
[20] Di Simplicio P, Jensson H, Mannervik B. Effects of inducers of drug metabolism on basic hepatic forms of mouse glutathione transferase. Biochem J 1989;263(3):679–85.
[21] Grubben MJ, Van Den Braak CC, Broekhuizen R, De Jong R, Van Rijt L, De Ruijter E, et al. The effect of unfiltered coffee on potential biomarkers for colonic cancer risk in healthy volunteers: a randomized trial. Aliment Pharmacol Ther 2000;14(9):1181–90.
[22] Scharf G, Prustomersky S, Huber WW. Elevation of glutathione levels by coffee components and its potential mechanisms. Adv Exp Med Biol 2001;500:535–9.
[23] Huber WW, Scharf G, Rossmanith W, Prustomersky S, Grasl-Kraupp B, Peter B, et al. The coffee components kahweol and cafestol induce gamma-glutamylcysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. Arch Toxicol 2002;75(11-12):685–94.
[24] Boekschoten MV, Schouten EG, Katan MB. Coffee bean extracts rich and poor in kahweol both give rise to elevation of liver enzymes in healthy volunteers. Nutr J 2004;3:7.
[25] Alvarez-Diez TM, Zheng J. Detection of glutathione conjugates derived from 4-ipomeanol metabolism in bile of rats by liquid chromatography-tandem mass spectrometry. Drug Metab Dispos 2004;32(12):1345–50.
[26] Kobayashi A, Kang MI, Watai Y, Tong KI, Shibata T, Uchida K, et al. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. Mol Cell Biol 2006;26(1):221–9.
[27] Copple IM, Goldring CE, Kitteringham NR, Park BK. The Nrf2-Keap1 defence pathway: role in protection against drug-induced toxicity. Toxicology 2008;246(1):24–33.
[28] Boerboom AM, Vermeulen M, van der Woude H, Bremer BI, Lee-Hilz YY, Kampman E, et al. Newly constructed stable reporter cell lines for mechanistic studies on electrophile-responsive element-mediated gene expression reveal a role for flavonoid planarity. Biochem Pharmacol 2006;72(2):217–26.
[29] Klett EL, Lu K, Kosters A, Vink E, Lee MH, Altenburg M, et al. A mouse model of sitosterolemia: absence of Abcg8/sterolin-2 results in failure to secrete biliary cholesterol. BMC Med 2004;2:5.
[30] Balani SK, Zhu T, Yang TJ, Liu Z, He B, Lee FW. Effective dosing regimen of 1-aminobenzotriazole for inhibition of antipyrine clearance in rats, dogs, and monkeys. Drug Metab Dispos 2002;30(10):1059–62.
[31] Yang KH, Choi YH, Lee U, Lee JH, Lee MG. Effects of cytochrome P450 inducers and inhibitors on the pharmacokinetics of intravenous furosemide in rats: involvement of CYP2C11, 2E1, 3A1 and 3A2 in furosemide metabolism. J Pharm Pharmacol 2009;61(1):47–54.
[32] Lee-Hilz YY, Boerboom AM, Westphal AH, Berkel WJ, Aarts JM, Rietjens IM. Pro-oxidant activity of flavonoids induces EpRE-mediated gene expression. Chem Res Toxicol 2006;19(11):1499–505.
[33] de Roos B, Caslake MJ, Stalenhoef AF, Bedford D, Demacker PN, Katan MB, et al. The coffee diterpene cafestol increases plasma triacylglycerol by increasing the production rate of large VLDL apolipoprotein B in healthy normolipidemic subjects. Am J Clin Nutr 2001;73(1):45–52.
[34] De Roos B, Van Tol A, Urgert R, Scheek LM, Van Gent T, Buytenhek R, et al. Consumption of French-press coffee raises cholesteryl ester transfer protein activity levels before LDL cholesterol in normolipidaemic subjects. J Intern Med 2000;248(3):211–6.
[35] Urgert R, Weusten-van der Wouw MP, Hovenier R, Meyboom S, Beynen AC, Katan MB. Diterpenes from coffee beans decrease serum levels of lipoprotein(a) in humans: results from four randomised controlled trials. Eur J Clin Nutr 1997;51(7):431–6.
[36] Post SM, de Wit EC, Princen HM. Cafestol, the cholesterol-raising factor in boiled coffee, suppresses bile acid synthesis by downregulation of cholesterol 7 alpha-hydroxylase and sterol 27-hydroxylase in rat hepatocytes. Arterioscler Thromb Vasc Biol 1997;17(11):3064–70.
[37] Chen LJ, DeRose EF, Burka LT. Metabolism of furans in vitro: ipomeanine and 4-ipomeanol. Chem Res Toxicol 2006;19(10):1320–9.
[38] Khojasteh-Bakht SC, Chen W, Koenigs LL, Peter RM, Nelson SD. Metabolism of (*R*)-(+)-pulegone and (*R*)-(+)-menthofuran by human liver cytochrome P-450s: evidence for formation of a furan epoxide. Drug Metab Dispos 1999;27(5):574–80.
[39] Peterson LA, Cummings ME, Vu CC, Matter BA. Glutathione trapping to measure microsomal oxidation of furan to cis-2-butene-1,4-dial. Drug Metab Dispos 2005;33(10):1453–8.
[40] Baer BR, Rettie AE, Henne KR. Bioactivation of 4-ipomeanol by CYP4B1: adduct characterization and evidence for an enedial intermediate. Chem Res Toxicol 2005;18(5):855–64.
[41] Cavin C, Mace K, Offord EA, Schilter B. Protective effects of coffee diterpenes against aflatoxin B1-induced genotoxicity: mechanisms in rat and human cells. Food Chem Toxicol 2001;39(6):549–56.
[42] Huber WW, Haslinger E, Schachner D, Parzefall W, Grasl-Kraupp B, Hochreiter A, et al. The carcinogen azonymethane causes necrosis and oxidative stress in rat liver within 48 hours after injection, a toxic effect that is however strongly mitigated by pretreatment with the coffee components kahweol and cafestol. Naunyn Schmiedebergs Arch Pharmacol 2007;375(Suppl 1):94.
[43] Urgert R, Meyboom S, Kuilman M, Rexwinkel H, Vissers MN, Klerk M, et al. Comparison of effect of cafetiere and filtered coffee on serum concentrations of liver aminotransferases and lipids: six month randomised controlled trial. BMJ 1996;313(7069):1362–6.
[44] Falkner KC, Pinaire JA, Xiao GH, Geoghegan TE, Prough RA. Regulation of the rat glutathione S-transferase A2 gene by glucocorticoids: involvement of both the glucocorticoid and pregnane X receptors. Mol Pharmacol 2001;60(3):611–9.
[45] Maglich JM, Stoltz CM, Goodwin B, Hawkins-Brown D, Moore JT, Kliewer SA. Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. Mol Pharmacol 2002;62(3):638–46.
[46] Tao KS, Wang W, Wang L, Cao DY, Li YQ, Wu SX, et al. The multifaceted mechanisms for coffee's anti-tumorigenic effect on liver. Med Hypotheses 2008;71(5):730–6.
[47] Surh YJ. NF-kappa B and Nrf2 as potential chemopreventive targets of some anti-inflammatory and antioxidative phytonutrients with anti-inflammatory and antioxidative activities. Asia Pac J Clin Nutr 2008;17(Suppl 1):269–72.
[48] Surh YJ, Na HK. NF-kappaB and Nrf2 as prime molecular targets for chemoprevention and cytoprotection with anti-inflammatory and antioxidant phytochemicals. Genes Nutr 2008;2(4):313–7.