Coffee Induces Expression of Glucuronosyltransferases by the Aryl Hydrocarbon Receptor and Nrf2 in Liver and Stomach

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BACKGROUND & AIMS: Coffee is one of the most widely consumed beverages worldwide. Epidemiologic data indicate that coffee consumption protects against the progression of chronic liver disease and development of hepatocellular carcinoma and diabetes, but the mechanisms are not clear. UDP glucuronosyltransferases (UGT1A) are proteins with indirect antioxidant, cytoprotective, and genoprotective capabilities; we examined *UGT1A* regulation in response to coffee in cultured cells and mice. **METHODS:** HepG2 and CaCo2 cells were incubated with regular, metal- or paper-filtered, decaffeinated, or instant coffee; green or black tea; cocoa; or metabolic products of caffeine. The effects of *UGT1A* regulation were investigated with reporter gene assays, immunoblot, TaqMan polymerase chain reaction, mutagenesis, and short interfering (si)RNA analyses. We also studied the effects of coffee in humanized transgenic mice that express human *UGT1A*. **RESULTS:** Incubation of cells with coffee induced transcription of *UGT1A1* (5.4-fold), *UGT1A3* (5.2-fold), *UGT1A4* (4.8-fold), *UGT1A7* (6.2-fold), *UGT1A8* (5.2-fold), *UGT1A9* (3.5-fold), and *UGT1A10* (6.1-fold). Induction was independent of caffeine, methylxanthines, or the diterpenes cafestol and kahweol. Mutagenesis and short interfering RNA knockdown studies showed that *UGT1A* is regulated by the aryl hydrocarbon receptor (AhR) and the nuclear factor erythroid-related factor 2 (Nrf2) by *cis*-acting antioxidant and xenobiotic response elements (ARE/XRE). In transgenic *UGT1A* mice, administration of coffee resulted in a 10- and 14-fold induction of *UGT1A* transcription in liver and stomach, respectively. **CONCLUSIONS: *UGT1A* genes are induced in vitro and in vivo by coffee, independent of caffeine content, cafestol, or kahweol. Coffee up-regulates glucuronidation by AhR signaling and Nrf2 binding to the ARE/XRE. Glucuronidation could mediate the protective and antioxidant effects of coffee.**

Keywords: Liver Cancer; Glucuronidation; Coffee.

Together with green tea, coffee is one of the most widely consumed beverages worldwide. The value of coffee beans as a commodity is only second to crude oil. Inhabitants of Western countries consume as much as 3 cups of coffee daily.[1] Coffee represents a readily available and much sought after caffeine delivery system because of the desired stimulatory effects on its consumers. However, apart from caffeine, coffee contains a plethora of complex organic compounds.[2] Coffee represents a rich source of phenols, polyphenols, flavanoids, and nonflavanoids, a number of which have been associated with antioxidant properties. A high proportion of chlorogenic acid and coffee bean flavanoids survive typical roasting temperatures of up to 230°C, but roasting also leads to mutagenic polyaromatic hydrocarbons.[2,3] Nevertheless, epidemiologic and study data suggest that coffee consumption is associated with a decreased risk of a number of diseases. In 1986, Arnesen et al[4] observed lower γ glutamyltransferase activities in coffee drinkers in the Tromso Heart study. This finding has been replicated in subsequent studies,[5] including an analysis of the third National Health and Nutrition Examination Survey (NHANES III) in 2005[6] that showed an inverse correlation of coffee intake and alanine aminotransferase activities. Coffee consumption has been associated with reduced risks of hepatocellular carcinoma (HCC),[7] liver cirrhosis,[8,9] and disease progression in chronic hepatitis C,[10] as well as with protective effects in Parkinson's disease[11] and type 2 diabetes,[12] and a controversial protection against colorectal cancer.[13]

To date, the responsible protective mechanisms of coffee remain to be fully elucidated. It has been suggested that caffeine may be responsible,[6] possibly by impaired transforming growth factor β signaling.[14] However, the role of caffeine alone was questioned by findings in other studies.[5,15,16] Coffee diterpenes (cafestol and kahweol) have been suggested to induce glutathione-*S*-transferases (GSTs) preventing benzo(α)pyrene genotoxicity,[17] and nuclear factor erythroid-related factor 2 (Nrf2)–mediated antioxidant action.[18]

Abbreviations used in this paper: ARE, antioxidant response element; HCC, hepatocellular carcinoma; Nrf2, nuclear factor erythroid-related factor 2; PCR, polymerase chain reaction; siRNA, short interfering RNA; *t*BHQ, *tert*-butylhydroquinone; TCDD, 2,3,7,8-tetrachlordibenzo-p-dioxin; UGT, UDP-glucuronosyltransferase; XRE, xenobiotic response element.

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On the basis of these data, we hypothesized that detoxification systems such as the UDP-glucuronosyltransferases (UGTs) may be regulated by coffee and thereby contribute toward cytoprotection and disease susceptibility. UGTs catalyze the formation of glucuronides from a broad array of potentially cytotoxic or genotoxic compounds,[19] which include human carcinogens and reactive oxygen species.[20–22] In addition, genetic UGT variants with reduced catalytic activity have been identified as risk factors for HCC and other cancers.[19,23–25] Regulation of UGTs by the xenobiotics present in roasted coffee would, therefore, represent a physiologically plausible mechanism of cytoprotection.

Material and Methods

Cell Culture Experiments

Hepatoma (HepG2), colon carcinoma (CaCo2), and esophagus carcinoma (KYSE70) cells were grown in RPMI 1640 (HepG2 and KYSE70) or Dulbecco's modified Eagle's medium with nonessential amino acids (CaCo2) supplemented with 10% fetal bovine serum.

Standardized Preparation of Coffee, Cocoa, and Tea

Stock solutions were prepared to represent the concentration and preparation mode of commonly used beverages: regular, decaffeinated, filtered, boiled, and instant coffee. For preparation of coffee and decaffeinated coffee, 150 mL of water (Aqua Irrigation Solution; DeltaSelect, Dreieich, Germany) was boiled in a beaker and cooled for 10 seconds. Six grams of ground coffee powder (Jacobs Krönung/Jacobs Krönung decaffeinated; Kraft Foods, Bremen, Germany) was added, incubated for 1 minute, and subsequently filtered through a paper coffee filter (Mellita, Minden, Germany). The undiluted filtrate was used for mice treatment, and diluted (12% coffee + 88% medium) filtrate was used for cell culture treatment. Two grams of Jacobs Krönung instant coffee (Kraft Foods) was solubilized in 150 mL of boiled water. For boiled coffee, 150 mL of water was boiled, cooled for 10 seconds, and 6 g of Jacobs Krönung coffee powder was added and incubated for 1 minute. The mixture was filtered through a metal sieve. Six grams of cocoa (Krüger, Bergisch Gladbach, Germany) was diluted in 150 mL of boiled water. For green tea, 150 mL of water were boiled and cooled for 2 minutes and 1 tea bag (green tea Meßmer, Seevetal, Germany) was incubated for 3 minutes. For preparation of black tea, 1 tea bag (black tea; Thiele Tee, Emden, Germany) was incubated in 150 mL of boiled water for 3 minutes. To show concentration dependency 1%, 4%, 8%, and 12% coffee stock solutions were used for luciferase experiments (data not shown). Induction was highest with 12%, which was used subsequently. As a control for filtered coffee 150 mL of water was boiled, cooled for 10 seconds, and paper filtered. For all other beverages water served as a negative control. Caffeine and theophylline concentrations were determined by enzyme-linked immunoabsorbent assay (Neogen, Lexington, KY; Table 1). The antioxidative capacities indicating polyphenol content were determined by an OxiSelect Oxygen Radical Antioxidant Capacity Activity Assay (Cell BioLabs, San Diego, CA).

Table 1. Chemical Composition of the Tested Beverages

	Caffeine (mg/cup, 150 mL)	Theophylline (mg/cup, 150 mL)
Coffee	95.8 ± 0.8	6.9 ± 0.5
Decaffeinated coffee	3 ± 0.3	0.6 ± 0.1
Instant coffee	93.4 ± 1.8	5.2 ± 0.4
Boiled coffee	128 ± 3.6	6.7 ± 1
Cocoa	14.5 ± 1	10.75 ± 0.5
Green tea	30.6 ± 0.8	2 ± 0.2
Black tea	37 ± 1.6	3.4 ± 0.3

Data are mean ± SD.

Methylxanthines and Coffee Lipids Cafestol and Kahweol

One cup of coffee following the above protocol contains approximately 100 mg of caffeine, which are metabolized to 70 mg of paraxanthine, 9 mg of theobromine, and 9 mg of theophylline.[26] Accordingly, these methylxanthine amounts (Sigma-Aldrich, Traufkirchen, Germany) were used per 150 mL of medium. Cafestol and kahweol (Sigma-Aldrich) were solubilized in dimethyl sulfoxide and diluted in medium to concentrations of 5, 30, and 56 μmol/L.

RNA Isolation and Reverse Transcription Polymerase Chain Reaction

HepG2, CaCo2, and KYSE70 cells were treated with coffee, tea, or cocoa solutions for 24 hours. Total RNA was isolated with TRIzol (Invitrogen, Karlsruhe, Germany). To isolate RNA from mouse organs, 100 mg of frozen tissue was homogenized in TRIzol. RNA (5 μg) was used for cDNA synthesis with the use of oligo(dT)-primed Superscript III reverse transcriptase (Invitrogen).

Quantitative Real-Time Polymerase Chain Reaction

By TaqMan, 0.2 μg of cDNA were analyzed (ABI Prism 7000 sequence detection system; Applied Biosystems, Foster City, CA) with the use of *UGT1A* isoform-specific primers and probes (Supplementary Table 1). Ct-values were normalized against β-actin.

Generation of Luciferase Reporter Gene Constructs

A 1000-base pair (bp) DNA fragment of the *UGT1A1* 5′-upstream region was amplified by polymerase chain reaction (PCR) from 2 healthy blood donors exhibiting the $A(TA)_6TAA$ or the $A(TA)_7TAA$ (UGT1A1*28) variant, respectively. The *UGT1A3* 258-bp DNA fragment and the *UGT1A4* 513-bp fragment were cloned from genomic DNA.[27,28] *UGT1A7* 5′ upstream DNA fragments