The Effects of Coffee on Conjugation Reactions in Human Colon Carcinoma Cells

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We examined the effect of coffee on conjugation reactions in the human colon carcinoma cell line, Caco-2. After supplementing Caco-2 cultures with both 1-naphthol (200 μM) and various concentrations of coffee, the accumulation of 1-naphthyl sulfate and glucuronide in the growth medium was determined by analytical HPLC over a 24-h period. A strong reduction in sulfo-conjugation (<50% of the control value) was observed in cells treated with coffee (IC50 = 4.3%), but no effect on glucuronic acid conjugation (glucuronidation) was observed. Coffee was also found to inhibit sulfotransferase (SULT) activity towards 1-naphthol in vitro to a similar extent (IC50 = 5.1%) as in intact Caco-2 cells, but exhibited no effect upon UDP-glucuronosyl transferase (UGT) activity in vitro. PCR analyses showed no significant changes in the expression of either SULT genes (SULT1A1 and SULT1A3) or UGT genes (UGT1A1 and UGT1A6) following treatment with coffee solutions of up to 5% in concentration. These results suggest that the consumption of coffee can modify sulfo-conjugation reactions within intestinal epithelial cells, which may possibly affect the bioavailability of therapeutic drugs and the toxicity of environmental chemicals.

Key words Caco-2; coffee; conjugation

Coffee is a widely consumed beverage and induces a series of metabolic and pharmacological effects, especially in the digestive tract. Several epidemiological studies have now shown that coffee consumption is associated with a lower risk of certain types of cancers, particularly colorectal cancers.1,2) In addition, evidence from a number of animal studies has provided further support for a potential chemoprotective effect of coffee; both coffee and constituents of coffee show protective effects against the action of specific carcinogens such as nitrosamines and 1,2-dimethylhydrazine.3,4) Several studies have also demonstrated the effects of coffee on hepatic enzymes involved in the detoxification of carcinogens, such as cytochrome P450 1A2 (CYP1A2), UDP-glucuronosyl transferases (UGTs) and glutathione S-transferases (GSTs).5—7) However, little is known about the effects of coffee on intestinal drug metabolizing enzymes.

Previously we have analyzed the effects of various types of tea, such as green, black and herbal teas, upon conjugation reactions in a human colon carcinoma cell line, Caco-2, and found that they show inhibitory activity, particularly against sulfo-conjugation reactions.8) Since sulfo-conjugation has been identified as a key step in the bioactivation of procarcinogens,9,20) it was therefore of interest to know whether coffee showed any effects upon similar conjugation pathways in the human digestive tract. Hence, we investigated the effects of coffee upon conjugation reactions in the Caco-2 cell line, which is a model of human intestinal epithelial cells.

MATERIALS AND METHODS

Materials All chemicals and reagents used in this study were of a HPLC analytical grade. Caffeine, 1-naphthyl sulfate, 1-naphthyl glucuronide and tetrabutylammonium hydrogen sulfate were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). Acetonitril was purchased from Wako Chemicals (Tokyo, Japan). Caco-2 cells were obtained at passage 40 from the RIKEN Cell Bank, Japan.

Preparation of Coffee Extract Blended coffee powder was obtained from Brooks Co., Japan. Coffee extract were prepared in a usual way in which 8 g of powder was extracted with 140 ml hot water (95 °C). The extract was then filtered and divided into small aliquots, and stored at −80 °C until use. Undiluted extract was assigned a concentration of 100% (v/v) with a dry weight of 8.4 mg/ml.

Cell Culture Caco-2 cells were grown in 12-well plates (Iwaki, Japan) in 1 ml MEM, supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 U/ml penicillin, 10 U/ml streptomycin and additional non-essential amino acids. The cells were seeded at a concentration of 5×103 cells/ml and grown until confluence (5—6 d) in a 37 °C incubator, in a humidified atmosphere containing 5% CO2. Cells were then further cultivated for up to three weeks. The media was changed every 4—5 d.

Analyses of 1-Naphthyl Sulfate and Glucuronide Quantitation of 1-naphthol conjugates was performed as described previously.9) Briefly, 1-naphthol (200 μM) was added to the medium, after which the cells continued to incubate at 37 °C. Aliquots (50 μl) of medium were then removed at various timepoints, and 30 μl of this mixture was filtered and injected into a HPLC apparatus equipped with an ODS column (Chromolith Performance RP-18e, 100×4.6 mm, Merck). The mobile phase consisted of 10 mM tetrabutylammonium hydrogen sulfate in water and acetonitril (72.5 : 27.5), and the flow rate was 1.0 ml/min with a column temperature of 30 °C. Elution was monitored at 285 nm. The retention times for 1-naphthol, 1-naphthyl sulfate and 1-naphthyl glucuronide were determined to be 11.4, 24.0 and 27.5 min, respectively. The linearity of the standard curves for 1-naphthol sulfate and glucuronide were observed up to 200 μM. The effects of coffee on conjugation reactions could then be measured by adding coffee to the culture medium of Caco-2 cells. IC50 values for the concentration–activity curves were calculated using a curve-fit program for Win-
dows.

Cytosolic Extract and Microsome Preparations from Caco-2 Cells Cells (1–2×10^5) were removed from the culture dishes (75 mm²), washed with phosphate buffered saline, and then homogenized in 1 ml buffer A (50 mM Tris–HCl (pH 7.5), 250 mM sucrose, 0.1 mM EDTA, 3 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml antipain, and 5 μg/ml pepstatin). The debris was removed by centrifugation at 3000 g for 15 min, after which the supernatant was centrifuged at 105000 g for 60 min. The clear lysate was used as the cytosolic extract, and the precipitate was used as the microsomal fraction in subsequent experiments.

Assay of P-ST Activity P-ST activity within the cytosolic fractions was determined using [35S]PAPS as the sulfate donor and 1-naphthol as the sulfate acceptor, according to a previously described, with slight modifications. Briefly, the reaction mixture (250 μl) contained 10 mM phosphate buffer (pH 7.4), 50 μM 1-naphthol, 5.0 μM [35S]PAPS (0.1 μCi) and cytosolic extract (50 μg of protein). The mixture was incubated at 37 °C for 30 min and the reaction was stopped by the addition of 50 μl cold 0.1 M barium acetate. Excess [35S]PAPS was precipitated by the addition of 50 μl of both 0.1 M Ba(OH)₂ and 0.1 M ZnSO₄ and removed by centrifugation at 12000 g for 5 min. This precipitation procedure was then repeated, and the remaining supernatant (300 μl) was transferred to a 3 ml liquid scintillator to determine radioactivity levels. Control reactions were established by omitting the acceptor substrate from the mixture.

Assay of UGT Activity UGT activity was determined as previously described, with slight modifications. Briefly, the reaction mixture (50 μl) contained 100 mM Tris–HCl (pH 7.4), 10 mM MgCl₂, 1 mM 1-naphthol and a microsome fraction (100 μg of protein). The reactions were initiated by the addition of 0.2 mM UDP-[U-14C]glucuronic acid (0.1 μCi) and continued for 30 min at 37 °C. The reactions were stopped by the addition of 100 μl ethanol (95%), and the mixtures were then centrifuged at 1000 g for 5 min. Aliquots (50 μl) were applied to silica-gel TLC plates (Merck 60F 254) and developed in n-butanol–acetic acid–glacial acetic acid–ammonia (30%)–water (70 : 50 : 18 : 1.5 : 60, v/v). Radioactive compounds were analyzed by a radioimage analyzer (Fuji Film, BAS2000).

RNA Isolation and Reverse Transcription-PCR (RT-PCR) Total RNA was isolated from cultured cells by the guanidium thiocyanate phenol–chloroform extraction method. First strand cDNA was synthesized from 10 μg of total RNA by 1 unit M-MLV reverse transcriptase with oligo(dT) primers, according to the manufacturer’s protocol. PCR was carried out, using this cDNA as a template, with AmpliTaq Gold polymerase (Perkin-Elmer). The PCR primers used to amplify human SULT and UGT cDNAs were designed from published sequences. The PCR reaction conditions (30–40 cycles) were as follows: 1 min at 94 °C, 1.5 min at 54–58 °C and 2 min at 72 °C.

Quantitative-PCR Quantitative real-time-PCR was performed with an ABI-Prism 7700 thermal cycler using a SYBR green PCR core reagent kit (Applied Biosystems, Warrington, U.K.). Samples were denatured at 94 °C for 10 min, and cDNA products were amplified with 40 cycles of denaturation at 94 °C for 30 s, then annealing and extension at 60 °C for 60 s. Calculations of the initial amounts of mRNA were performed according to the cycle threshold method. The mRNA levels were normalized to β-actin levels, which had been quantified by RT-PCR.

RESULTS AND DISCUSSION

Effects of Coffee on Conjugation Reactions in Caco-2 Cells To determine the effects of coffee on conjugation reactions in intact Caco-2 cells, we measured the sulfo- and glucuronic acid conjugates of 1-naphthol in the cellular growth media which had been supplemented with 200 μM 1-naphthol in the presence or absence of coffee, as described previously. As shown in Fig. 1A, 1-naphthyl sulfate and 1-naphthyl glucuronide levels accumulate in the culture medium in a manner that is almost directly proportional to the incubation time. After 24 h of culture, more than 80% of the initial 1-naphthol supplement had been converted to either its sulfo- or glucuronic acid conjugate forms (data not shown). Following the addition of coffee into the medium (up to a concentration of 5%), a decrease in sulfo-conjugation was observed in a dose-dependent manner (IC₅₀=4.3%: 0.36 mg extract/ml), whereas no detectable changes were detected in glucuronidation activity (Fig. 1A).

As caffeine is a major component of coffee, and it has many physiological properties, we also analyzed its effects on conjugation activities in Caco-2 cells. Caffeine was found, however, to show only a weak inhibitory effect on sulfo-conjugation and an even lesser effect on glucuronidation in Caco-2 cells at a dose of 100 μM (Fig. 1B). The concentration of caffeine in the coffee extract was calculated to be 1–2 mM based upon previously published data, demonstrating that 5–10% of the total constituents of coffee are in fact caffeine. Hence, 100 μM caffeine is equivalent to an approximately 10–20% solution of coffee extract. From these data we conclude that caffeine is not the component in coffee that is responsible for its inhibitory action on conjugation reactions.

Effects of Coffee on SULT and UGT Activities in Vitro To elucidate the underlying mechanism(s) by which coffee affects conjugation activities in Caco-2 cells, we next measured its effects on the enzymatic activities of SULT and UGT towards 1-naphthol in vitro. As shown in Fig. 2A, exposure to coffee strongly inhibits in vitro SULT activity (IC₅₀=5.1%: 0.43 mg extract/ml), but shows no significant effects on UGT activity. The IC₅₀ values in these cases were almost identical to those measured in intact cells, suggesting that the inhibitory effect of coffee on the sulfo-conjugation of 1-naphthol in Caco-2 cells might be due to direct interaction with the enzyme proteins inside the cell. Further analysis will need to be undertaken to clarify the molecular basis of this inhibition.

Effects of Coffee on SULT and UGT Gene Expression As recent studies have demonstrated that coffee components can affect several drug metabolizing enzymes in mammalian cells by regulation of their gene expression, we investigated the effects of coffee on the expression of both SULT and UGT genes. In humans, several subfamilies of SULT and UGT genes have now been identified, and among these we selected SULT1A1, SULT1A3, UGT1A1 and UGT1A6, due to their substrate specificities. The expression levels of each
of these genes was subsequently monitored by RT-PCR after treatment with 5% coffee for 24 h. As shown in Fig. 3, no obvious changes were detected in the levels of SULT or UGT expression tested, and this was confirmed by quantitative real-time PCR analysis (data not shown).

Recent studies have demonstrated the induction of different isoforms of the UGT1A subfamily in Caco-2 cells by dietary flavonoids, such as chrysin and quercetin. Coffee also contains several phenolic compounds with antioxidant properties, and its total polyphenol content has been reported to range from 200 to 550 mg per serving. Such phenolic antioxidant compounds in coffee may induce UGT1A6 expression in Caco-2 cells, but no induction of UGT1A1 or UGT1A6 genes was observed in our experiments. It is possible, however, that the incubation periods (24 h) adopted in our experiments were not sufficiently long to induce UGT1A gene expression.

In this report we describe the effects of coffee on conjugation reactions in a human intestinal model cell line, Caco-2. We demonstrate that coffee strongly inhibits sulfo-conjugation in these cells, whereas caffeine, a major component of coffee, shows little significant effect on SULT activity either in vivo or in vitro. Neither coffee nor caffeine, therefore, show any effects on UGT activity in Caco-2 cells. It is currently uncertain which of the components of coffee is in fact responsible for the inhibition of SULT activity but phenolic components are the most likely candidates due to the substrate specificity of SULTs. As sulfo-conjugation has been shown to be responsible for the bioactivation of proximal carcinogens in many studies, this inhibitory feature of coffee may be related to the observed anticancer effects of coffee consumption. Additional characterizations should...
therefore be carried out to further clarify the molecular basis of the effects of coffee on conjugation reactions.

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REFERENCES