Coffee Reduces SULT1E1 Expression in Human Colon Carcinoma Caco-2 Cells

Marina Isshiki, Haruka Ohta, and Hiroomi Tamura*

Graduate School of Pharmaceutical Sciences, Keio University; Minato-ku, Tokyo 105–8512, Japan.
Received October 17, 2012; accepted November 19, 2012

Recent epidemiological studies have shown that moderate coffee consumption is associated with a lower risk of certain types of cancers, particularly colon cancer in postmenopausal women. To elucidate the molecular basis for the preventive action of coffee, we investigated the effect of coffee on estrogen sulfotransferase (SULT) because sulfation is the major pathway involved in the inactivation of estrogens. We found that coffee reduced SULT1E1 gene expression in human colon carcinoma Caco-2 cells. Treatment with 2.5% (v/v) coffee for 24 h resulted in a 60% reduction of the expression of the SULT1E1 gene in Caco-2 cells. Corresponding to reduced SULT1E1 gene expression, cytosolic estrogen SULT activity toward E2 (20 nM) decreased by 25%. In addition, an accumulation of E2 sulfates in the medium, which reflects cellular activity of estrogen SULT, decreased after the cells were treated with coffee. Major bioactive constituents in coffee such as caffeine, chlorogenic acid and caffeic acid did not show any effect. The inhibitory activity was extractable by using ethyl acetate. We also found that the inhibitory activity was produced by roasting the coffee beans. Mithramycin, an inhibitor of the transcription factor stimulating protein 1 (Sp1), was able to restore coffee-reduced SULT1E1 gene expression. Our data suggest that daily coffee consumption may reduce estrogen SULT activity, thereby enhancing estrogenic activity in the colon.

Key words Caco-2; coffee; colon cancer; estrogen; SULT1E1; stimulating protein 1

Materials and Methods

Materials The reagents for polymerase chain reaction (PCR) were obtained from Applied Biosystems, Inc. (Warrington, U.K.). Caco-2 cells at passage 40 were obtained from the RIKEN Cell Bank (Tokyo, Japan). Columbian Arabica coffee beans were purchased from Nakaya Coffee (Tokyo, Japan).

Cell Culture Caco-2 cells were grown in 6-well plates (Iwaki, Tokyo, Japan) in 2 mL of minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 U/mL penicillin, 10 U/mL streptomycin, and additional non-essential amino acids. Cells were seeded at a concentration of 5 × 10^5 cells/mL and grown to confluence (5–6 d) in an incubator at 37°C humidified atmosphere containing 5% CO2. Subsequently, the cells were further cultivated for up to 3 weeks for functional differentiation. The medium was changed every 4–5 d.

Preparation of Coffee Extract Blended coffee powder (Arabica beans) was obtained from Starbucks Coffee Japan (Tokyo, Japan). Coffee extracts were prepared using a common method in which 8 g of powder was extracted with 140 mL of hot water (95°C). The extract was then filtered, divided into small aliquots, and stored at −80°C until used. Undiluted extract, with a dry weight of 8.4 mg/mL, was assigned a concentration of 100% (v/v).

Oz 2013 The Pharmaceutical Society of Japan

©

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: tamura-hr@pha.keio.ac.jp

© 2013 The Pharmaceutical Society of Japan

The authors declare no conflict of interest.
Roasting of Coffee Beans

 Colombian Arabica coffee beans were roasted in a roaster at 220°C for 5, 10, 15, 20 and 25 min. Coffee beans at each roasting time were pulverized, and 8 g of the sample was extracted with 140 mL of boiling water.

Analysis of Gene Expression

Total RNA was isolated from the cultured cells by using the guanidine thiocyanate-phenol-chloroform extraction. First strand cDNA was synthesized from 10 μg of total RNA by using 1 unit of M-MLV reverse transcriptase with oligo (dT) primers, according to the manufacturer’s protocol. PCR was performed with Taq DNA polymerase using this cDNA as a template. Primers used for the amplification of cDNAs were designed based on published sequences as follows (forward and reverse): 5′-TTG CCA CCT GAA CTT CCT CCT GCC-3′ and 5′-TTG GAT GACC CAG CCA CCA TTA GAA-3′. Quantitative real-time PCR was performed in an ABI 7300 thermal cycler using a SYBR green PCR core reagent kit (Applied Biosystems Inc., Warrington, U.K.). Samples were denatured at 94°C for 10 min, and cDNA products were then amplified using 40 cycles of denaturation at 94°C for 30 s followed by annealing and extension at 60°C for 60 s. The amount of target gene relative to a reference gene (18S RNA) was quantified using the cycle threshold (Ct).

Cytosolic Extract Preparation from Caco-2 Cells

Caco-2 cells (1–2×10^5) were removed from 75-mm² culture dishes, washed in phosphate buffered saline (PBS), and then homogenized in 1 mL of buffer A (50 mM Tris–HCl pH 7.5, 250 mM sucrose, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 3 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/mL antipain, and 5 μg/mL pepstatin). Cell debris was removed via 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 in subsequent experiments.

E₂ Sulfation Assays

For studies of E₂ sulfation in intact Caco-2 cells, coffee-treated cells were washed in PBS and incubated with 20 mM [³¹H]E₂ in MEM supplemented with 5% charcoal-treated fetal bovine serum at 37°C. Next, 50-μL aliquots of the medium were sampled at various times and E₂ sulfate formation was measured using the alkaline–chloroform extraction (Fig. 1). The amount of target gene relative to a reference gene (18S RNA) was quantified using the cycle threshold (Ct).

Bromide (MTT) Assay

Cytotoxic effects on cell metabolism were investigated using the MTT assay. Cells (1×10^4) were seeded in 96-well plates and grown for 3 weeks at 37°C. After incubation with coffee at various concentrations, the cells were washed with PBS and subjected to the MTT assay according to the manufacturer’s protocol (Dojin Chemicals, Tokyo, Japan).

Statistical Analysis

Data were statistically analyzed using Dunnett’s multiple comparison tests. Results were considered significant when p values were less than 0.05.

RESULTS

Effects of Coffee on SULT1E1 Gene Expression

Caco-2 cells were incubated with coffee for 24 h, and then SULT1E1 gene expression was examined using real-time PCR (Fig. 1). With 2.5% coffee, the expression of SULT1E1 gene was reduced to 40% of the control. Changes in the gene expression were detected at an early stage of culture (6 h, data not shown). No cytotoxicity was observed with up to 10% (v/v) coffee as assessed using the MTT assay (data not shown).

Using E₂ at physiological concentrations (10–100 nM) as a substrate, it is possible to measure estrogen SULT activity catalyzed by SULT1E1 in Caco-2 cells as previously reported. To evaluate the effect of coffee on the SULT1E1 gene expression in Caco-2 cells, cytosolic estrogen SULT activity of coffee-treated cells was measured in vitro (Fig. 2A). Estrogen SULT activity in the cytosol of coffee-treated cells decreased with increasing coffee concentration. Furthermore, an accumulation of E₂ sulfate in the medium from cells treated with 2.5% coffee was 25% lower than the control (Fig. 2B).

Chemical Properties of Active Coffee Compounds

There are several major constituents in coffee extracts, which exhibit specific physiological activities. These include caffeine, chlorogenic acid, and caffeic acid. As shown in Fig. 2A, these compounds at 100 μM showed no significant effects on SULT1E1 gene expression in Caco-2 cells. This concentration is almost equivalent to that in 10–50% coffee for each compound. To further characterize the active constituents in coffee, brewed coffee was sequentially extracted with an equal
volume of solvents (n-hexane, ethyl acetate, chloroform, and n-butanol), and the effect of each extract was then measured. Inhibitory activity was recovered mainly in the ethyl acetate extract, and a slight residual activity was detected in the n-hexane extract (Fig. 3B).

**Effect of Roasting on the Inhibitory Activity in Coffee** To examine the possibility that active components are formed during the roasting process, we assayed the activity of extracts of green beans that had undergone varying degrees of roasting before brewing. We extracted the beans roasted for 0–20 min and applied the extracts for the assays. Increasing the degree of roasting resulted in an increased ability to reduce the SULT1E1 gene expression (Fig. 4). The inhibitory activity appeared after the beans had been roasted for 10 min.

**Involvement of Sp1 in Coffee-Mediated Changes in SULT1E1 Gene Expression** Previously we demonstrated that coffee induced BCRP gene expression in Caco-2 cells via NF-κB activation\(^\text{10}\); however, no obvious effects were observed with an NF-κB inhibitor, DHMEQ, on the coffee-mediated reduction of SULT1E1 gene expression (data not shown). Because studies show that the transcription factor stimulating protein 1 (Sp1) regulates SULT1A1 and SULT2B1b gene expression,\(^\text{15,16}\) we investigated the possible involvement of Sp1 in coffee-mediated reduction of SULT1E1 gene expression. For this purpose, we measured the effect of mithramycin (Mit), an Sp1 inhibitor.\(^\text{17}\) As shown in Fig. 5, Mit diminished the reductive effect of coffee, and furthermore induced significant levels of SULT1E1 gene expression.

**DISCUSSION**

We demonstrated here that constituents in brewed coffee reduced SULT1E1 gene expression and thereby SULT1E1 activity in human colon carcinoma Caco-2 cells (Figs. 1, 2).
For our knowledge, this is the first report showing the reduction of SULT1E1 gene expression by coffee. By 2.5% coffee, the expression of the SULT1E1 gene was reduced to less than 50% of the control, however, with lower concentrations below 1.25% the levels of the reduction in SULT1E1 gene expression by coffee varied among experiments (data not shown). This might be due to low concentrations of active components. To clarify this issue, we are now trying to isolate active components from the coffee extract. As compared to the effect on the gene expression, the effects on the estrogen SULT activity was relatively low (Fig. 2). This might be due to a slower rate of degradation of the protein with compared to that of mRNA. This possibility should be clarified in future.

Most active components, with the exception of the major components caffeine, chlorogenic acid, and caffeic acid, are extractable using ethyl acetate (Fig. 3). Weak inhibitory activities detected in other solvent fractions indicate the existence of several other compounds with different molecular properties that have inhibitory activity toward SULT1E1 gene expression. Further analysis is needed to clarify this observation.

We found that active components are formed during the roasting process (Fig. 4). After roasting for 10 min 220°C, the inhibitory activity appeared, and it was maintained. Many phenolic constituents and Maillard reaction products are known to form during the coffee roasting process and are extractable with ethyl acetate.18–20 These compounds are reported to possess various types of antioxidant and pro-oxidant activity,21,22 which often modulate antioxidant transcription factors such as NF-κB and nuclear factor-E2-related factor 2 (Nrf2). Indeed, we have observed coffee-induced NF-κB-mediated activation of the BCRP gene in Caco-2 cells.10 However, DHMEQ, an inhibitor for NF-κB, had no effect on coffee-mediated effects on SULT1E1 gene expression (data not shown).
shown). Using Mit, an Sp1 inhibitor, we demonstrated that the reduction in SULT1E1 gene expression by coffee might be mediated by Sp1 (Fig. 5). Interestingly, Mit alone induced SULT1E1 gene expression in Caco-2 cells. This suggests that in normal states, expression of the SULT1E1 gene might be repressed by Sp1 in Caco-2 cells. Our preliminary analysis of the promoter region of the SULT1E1 gene identified the presence of a possible Sp1 binding site 3.25 kb upstream of the transcription initiation site (data not shown). Precise analysis of regions necessary for coffee-mediated-reduction of SULT1E1 gene expression is now under investigation in our laboratory.

Sulfonation plays a significant role in the metabolism of estrogens and contributes to the maintenance of circulating levels of these hormones. In particular, the intracellular metabolism of estrogens is important for the bioformation and the activity of E2. Under physiological conditions, E2 is primarily sulfated by SULT1E1, which is expressed in many human tissues including liver, jejunum, mammary epithelial cells, endometrium, and testis. Several studies have reported that dietary flavonoids such as quercetin and resveratrol inhibit estrogen-mediated SULT activity in human mammary epithelial cells, as well as in hepatic and jejunal S9 fractions. We also reported that among the different beverages tested, coffee in particular inhibited the SULT1E1 activity in Caco-2 cells. These effects of food constituents therefore affect the intracellular availability of estrogen hormones to their receptors and influence the progression of estrogen-sensitive cancers. As shown in Fig. 2B, coffee treatment reduced the intracellular activity for E2 sulfation. This change may affect the level of active estrogens within the cells. We speculate that this change may be linked to results from epidemiological studies indicating that coffee consumption, especially for postmenopausal women, results in a reduction of colon cancer risk. Currently, it is unclear how estrogens reduce the risk of colon cancer in postmenopausal women. Recent studies have demonstrated that the estrogen receptor β (ERβ) is the predominantly expressed ER in the colon, and loss of ERβ in colorectal cancer has been associated with advanced cancer stages. Elevated intracellular estrogen levels induced by daily coffee consumption may adversely influence the progression of colorectal cancer in postmenopausal women. Further investigation of the molecular mechanisms underlying this phenomenon is needed.

Acknowledgments We thank Dr. Kitaro Oka for his encouragement. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES


