Effect of Herbal Teas on Conjugation Reactions in a Human Colon Carcinoma Cell Line, Caco-2

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We examined the effect of herbal teas (including black and green teas) on conjugation reactions within a human colon carcinoma cell line, Caco-2. After adding herbal tea to the culture medium of Caco-2 cells, accumulation of 1-naphthyl sulfate and glucuronide was determined within the medium by analytical HPLC prior to 24 hr. A reduction in sulfoconjugation (< 50% of the control value) was observed in cells treated with 5% solutions of green tea, jasmine tea or black tea. Mild induction of sulfoconjugation (20–30%) was detected in cells exposed to 5% solutions of hibiscus or lemongrass tea. In addition, a slight reduction (20–30%) in glucuronic acid conjugation (glucuronidation) was observed in cells treated with rose red, peppermint, lavender or St. John's wort. At low concentrations, ranging from 0.1–1.0%, several herbal teas resulted in weak but significant induction (10–20%) of both types of conjugation reactions. These results suggest that herbal teas modify conjugation reactions within intestinal epithelial cells, thereby potentially affecting the bioavailability and toxicity of therapeutic drugs and environmental chemicals.

Key words —— Caco-2, glucuronidation, herbal tea, sulfoconjugation

INTRODUCTION

Herbal teas, which are made from the leaves, flowers and other parts of various herbs, are consumed by millions of people around the world. Herbal teas are generally used as dietary supplements and traditional medicines. They have made a significant contribution to human health for thousands of years and have received a lot of attention in recent years as alternative medicines.[1] Since they are often administered in combination with conventional therapeutic drugs, it is very important to explore potential herb-drug interactions.[2] A recent study has examined the effects of herbal teas on the activity of rat hepatic phase I and phase II drug metabolizing enzymes.[3] This study has demonstrated suppression of CYP1A2 activity and induction of UDP-glucuronosyl transferase by several herbal teas. Although drug metabolism primarily occurs in the liver, intestinal metabolism is crucial to ensure bioavailability of orally administered drugs and environmental chemicals. Previously, we have studied sulfoconjugation reactions in Caco-2 cells from a human colon carcinoma cell line and have observed inhibitory effects of catechins (green tea components) on sulfoconjugation.[4] To further identify potential herb-drug interactions, particularly those which might affect intestinal metabolism, we examined the effect of herbal teas on conjugation reactions (sulfoconjugation and glucuronidation) in Caco-2 cells.

MATERIALS AND METHODS

Materials —— All chemicals and reagents used were of HPLC analytical grade. 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). The leaves and dried flower heads used to extract the herbal teas were purchased from Japan Green Tea Center (Tokyo, Japan). Caco-2 cells were obtained at passage 40 from RIKEN Cell Bank, Japan. **Preparation of Herbal Tea Extracts** —— Herbal tea solutions were prepared simulating the usual way of brewing tea. Leaves or dried flower heads of herbs (2 g) were extracted with 100 ml of hot water (75°C) for 2 min. The extract was then filtered and divided into small aliquots, after which it was stored at –80°C

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until use. Undiluted extract was assigned a concentration of 100% (v/v).

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 kept at 37°C in a humidified atmosphere, containing 5% CO_2. The cells were seeded in 12-well plates at a concentration of 5×10^5 cells/ml and grown until confluence (5–6 days). The cells were cultivated for up to 2 weeks and their media was changed every 4–5 days.

Analyses of 1-Naphthyl Sulfate and Glucuronide —— Quantitation of 1-napthol conjugates was performed as described previously.[4] In brief, 1-Naphthol (200 μM) was added to the medium, after which the cells were incubated at 37°C. Aliquots (50 μl) were removed at various times, after which 30 μl of the mixture was filtered and injected into the HPLC apparatus, equipped with an ODS column (CAPCELL PAK C_{18}UG80, 250 × 4.5 mm, Shiseido, Japan). The mobile phase consisted of 2 mM tetrabutylammonium hydrogen sulfate in water and acetonitril (65 : 35). The flow rate was 1.0 ml/min with a column temperature of 40°C. Elution was monitored at 285 nm. The retention times for 1-naphthol, 1-naphthyl sulfate and 1-naphthyl glucuronide were determined to be 19.0 min, 17.6 min and 4.6 min, respectively. Linearity of the standard curves for 1-naphthyl sulfate and glucuronide was observed up to 200 μM. The effects of herbal teas on conjugation reactions were thus measured by adding herbal teas to the culture medium of Caco-2 cells. IC_{50} values for the concentration-activity curves were calculated using a curve-fit program for Windows.

RESULTS AND DISCUSSION

Previously, we reported on the sulfoconjugation of 1-naphthol in intact Caco-2 cells.[4] In this study, we extended our analysis to investigate glucuronidation of 1-naphthol in Caco-2 cells. Both conjugation reactions were detected in Caco-2 cells in a substrate dose- and culture time- dependent manner prior to 24 hr (data not shown).

To explore the potential for herb-drug metabolism interactions, specifically those involving conjugation reactions, we directly added herbal teas to
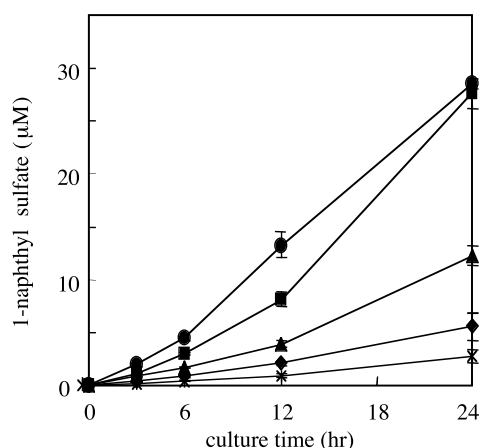

Fig. 1. Effect of Green Tea on Sulfoconjugation Reaction in Caco-2 Cells

After addition of green tea at different concentrations with 1-naphthol (200 μM), cells were cultured for indicated times and the concentrations of 1-naphthyl sulfate were determined (●, 0%; ■, 0.5%; ▲, 1.0%; ◆, 2.5%; ×, 5%). All points represent means of three determinations with S.D.

the culture medium, along with 200 μM of 1-naphthol, after which the accumulation of conjugation products was measured within the medium prior to 24 hr. Fig. 1 shows the representative results. Fig. 2 summarizes the results of 15 herbal teas administered in concentration of 5%.

A marked reduction in sulfoconjugation (< 50% of the control value) was observed following exposure to 5% solutions of green tea, jasmine tea and black tea (Fig. 2). Moderate inhibition of sulfoconjugation was observed following exposure to rose red, oolong, chamomile, peppermint and lavender teas (Fig. 2). The IC_{50} values of herbal teas that inhibited sulfation of 1-naphthol are shown in Table 1. In general, teas made from *Camellia sinensis* showed strong inhibition, suggesting that catechins may be involved in the inhibition. This is supported by our finding in previous studies that epigallocatechin gallate (EGCG) is strong inhibitor of sulfoconjugation in Caco-2 cells.[4,5] Mild induction (20–30%) of sulfoconjugation was observed in Caco-2 cells following exposure to 5% hibiscus or lemongrass tea. Interestingly, low concentrations (0.1–1.0%) of hibiscus and lemongrass teas had the greatest inductive effect (Fig. 3). Although inhibition was observed at concentrations of 5%, several herbal teas, such as green tea, the results for which are shown in Fig. 3, induced sulfoconjugation at low concentrations (0.05–0.5%).

Despite the observed changes in sulfo-conjuga-
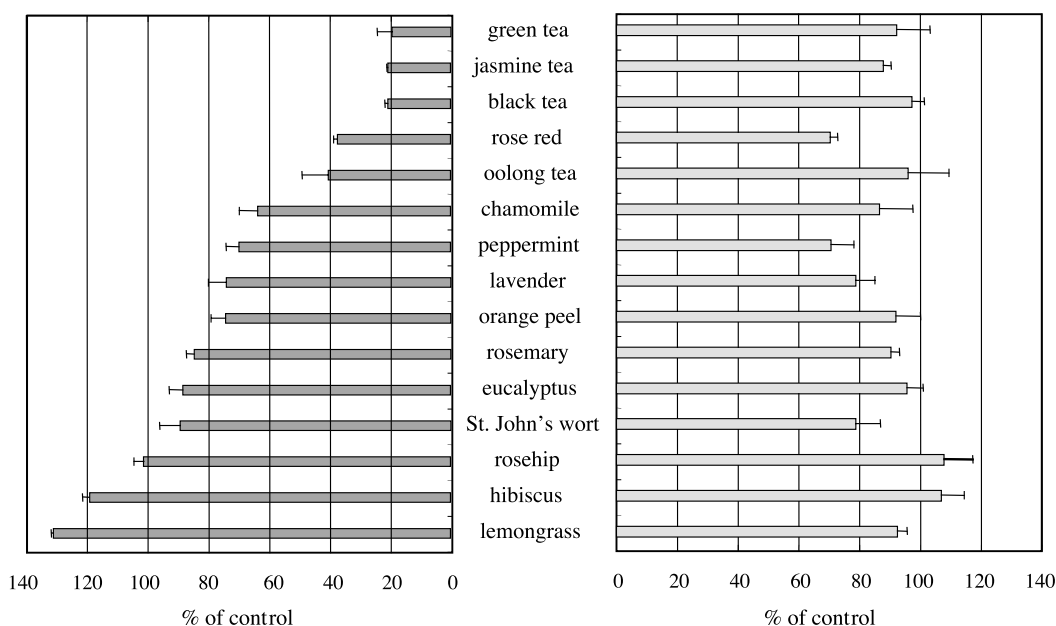
Fig. 2. Effects of 5% Herbal Teas on Conjugation Reactions in Caco-2 Cells

1-Naphthyl sulfate and glucuronide in the culture medium were measured at 24 hr after addition of 5% herbal teas and 200 μM 1-naphthol. Left, 1-naphthyl sulfate; right, 1-naphthyl glucuronide. Percentages of the control (=100) were calculated. All values are means of three determinations with S.D.

tion, little change in glucuronidation was observed following exposure of Caco-2 cells to 5% solutions of the various herbal teas. A slight reduction (20–30%) in glucuronidation was observed in cells treated with extracts of rose red, peppermint, lavender and St. John's wort (Fig. 2). Interestingly, low doses (0.1–1%) of herbal teas such as green tea, black tea, rosehip, and eucalyptus, induced glucuronidation (10–20%) (Fig. 3 shows the results for rosehip). A recent study has reported induction of hepatic UDP-glucuronosyl transferase (UGT) in rats administered peppermint or chamomile tea for 2 weeks (70–80%),[3] however, we observed mild inhibition of glucuronidation in Caco-2 cells treated with these same herbal teas. The difference in these results might be explained by the use of different substrates (*p*-nitrophenol *vs* 1-naphthol), tissues (liver *vs* intestine) and systems (*in vivo vs* intact cells).

Here we report that herbal teas alter conjugation reactions in Caco-2 cells. The mechanism by which herbal teas modulate conjugation reactions in Caco-2 cells is not clear at the present time. A simple explanation is that components of the herbal teas might interact directly with sulfotransferase and/or UGT enzymes within the cells. In fact, we have previously shown that catechins and other flavonoids, the two major components of herbal teas, inhibit intestinal phenol sulfotransferase (P-ST) activities *in vitro*.[5,6] However, evidence regarding the *in vitro* effects of

Table 1. IC$_{50}$ Values of Herbal Teas Showing Inhibitory Activity on the Sulfoconjugation Reaction in Caco-2 Cells

Herbal teas	IC$_{50}$ (%)
jasmine tea	$1.2 \pm 0.1^{a)}$
green tea	2.4 ± 0.1
black tea	2.4 ± 0.3
rose red	2.8 ± 0.3
oolong tea	3.1 ± 0.2
lavender	7.9 ± 0.5
peppermint	8.5 ± 1.2

$a)$ $n = 3$.

herbal teas on UGT activity is lacking to date. Another explanation for the ability of herbal teas to modify conjugation reactions might be regulation of gene expression at a transcriptional and/or translational level. Recent reports have shown that dietary flavonoids such as chrysin, quercetin and genistein can induce UGT activity.[7,8] Induction of UGT1A1 by flavonoids has been shown to occur at a transcriptional level.[8] However, a preliminary analysis performed by our laboratory observed no obvious changes in the mRNA levels of UGTs (UGT1A and UGT2B families) or sulfotransferases (SULT1A family). Another possible mechanism is regulation of transport activity. There is accumulating evidence to suggest that flavonoids and other herbal components might alter the activity of vari-

(A)   (B)
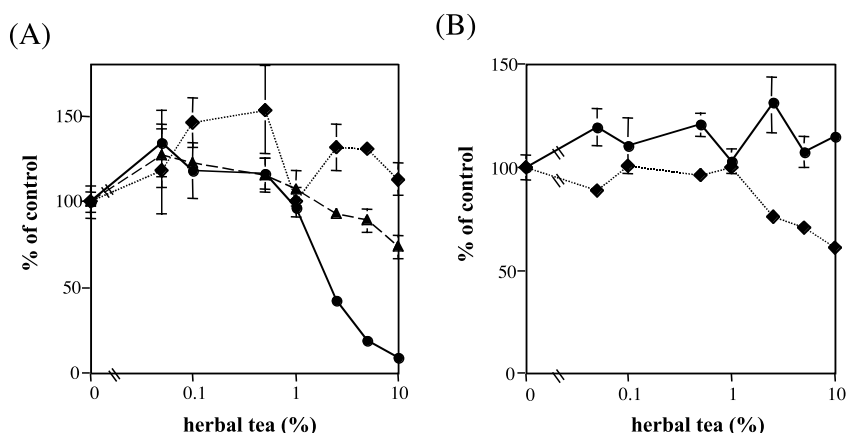
Fig. 3. Dose Responses of Herbal Teas on 1-Naphthyl Sulfate (A) and 1-Naphthyl Glucuronide (B) Accumulations in the Culture Medium of Caco-2 Cells

Representative results are shown. (A) green tea, ●; lemongrass, ◆; St. John's wort, ▲; (B) rosehip, ●; peppermint, ◆. All values represent the means of three determinations with S.D.

ous transporters.[9–11] Studies examining the effects of herbal teas on various transporters are now progressing in our laboratory. Since diet-mediated induction or inhibition of intestinal conjugation reactions has implications with regard to the bioavailability of carcinogens and other toxic chemicals, as well as therapeutic drugs, further study is required to elucidate the mechanism by which herbal teas alter conjugation reactions.

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