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

Shigeaki Okamura and Hiro-omi Tamura*

Kyoritsu University of Pharmacy, Shibakoen, Minato-ku, Tokyo 105–8512, Japan

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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.
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% CO₂. The cells were seeded in 12-well plates at a concentration of 5 × 10⁵ 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 C₁₈ 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, 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 IC₅₀ 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 induc-
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%), but 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).

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

<table>
<thead>
<tr>
<th>Herbal teas</th>
<th>IC_{50} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>jasmine tea</td>
<td>1.2 ± 0.1²</td>
</tr>
<tr>
<td>green tea</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>black tea</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>rose red</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>oolong tea</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>lavender</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>peppermint</td>
<td>8.5 ± 1.2</td>
</tr>
</tbody>
</table>

² n = 3.

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. However, evidence regarding the in vitro effects of 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. Induction of UGT1A1 by flavonoids has been shown to occur at a transcriptional level. 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-

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.
ous transporters.\textsuperscript{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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REFERENCES


