

## Turmeric and Curcumin Modulate the Conjugation of 1-Naphthol in Caco-2 Cells

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**Turmeric, the powdered dry rhizome of the *Curcuma longa* plant, and curcumin, the major anti-oxidant constituent of turmeric, have been shown to possess chemopreventive activity. To elucidate the possible interaction of turmeric and curcumin with conjugation reactions, which in many cases are involved in the activation of procarcinogens, we measured their effects in the conjugation of 1-naphthol in Caco-2 cells, a human colon carcinoma cell line, within a 24 h period. Turmeric exhibits inhibitory activity toward both sulfo- and glucuronosyl conjugations of 1-naphthol at approximately the same levels ( $IC_{50}$ =0.24 and 0.29 mg/ml, respectively). Curcumin inhibits sulfo-conjugation at lower concentrations ( $IC_{50}$ =9.7  $\mu$ g/ml), but only showed weak inhibition toward glucuronosyl conjugation of 1-naphthol in Caco-2 cells. In addition, turmeric was found to strongly inhibit *in vitro* phenol sulfotransferase (SULT) activity and demonstrate moderate inhibitory properties against UDP-glucuronosyl transferase (UGT) activity in Caco-2 cells ( $IC_{50}$ =0.17 mg/ml and 0.62 mg/ml, respectively). Curcumin also strongly inhibits *in vitro* phenol sulfotransferase activity with an  $IC_{50}$  of 2.4  $\mu$ g/ml. Moreover, and in contrast to the moderate inhibition of UGT activity by turmeric and curcumin, both induce the expression of the UGT1A1 and UGT1A6 genes, revealed by real-time PCR analysis. These findings are indicative of a possible interaction of both turmeric and curcumin with conjugation reactions in the human intestinal tract and colon. This in turn may affect the bioavailability of therapeutic drugs and toxicity levels of environmental chemicals, particularly procarcinogens.**

**Key words** Caco-2; conjugation; curcumin; sulfotransferase (SULT); turmeric; UDP-glucuronosyl transferase (UGT)

Turmeric is the powdered dry rhizome of the plant *Curcuma longa* and has been widely used as a coloring agent, as a spice and has been utilized in the treatment of inflammatory conditions and other diseases.<sup>1)</sup> Curcumin is a yellow pigment and is the major anti-oxidant and anti-inflammatory constituent of turmeric. It is present in curry and mustard, and it is used extensively in Asian countries and in traditional medicines. Curcumin and/or turmeric have both been shown to possess cancer chemopreventive activity in addition to anti-inflammatory activity, and these compounds have thus generated considerable attention as alternative medicines in recent years.<sup>2-4)</sup> The low incidence of colon cancer in Asian countries could be related to low meat intake, but may also be due to the regular use of turmeric in the diet of these regions. As the colon is exposed to turmeric and curcumin, it is a likely target for the anticarcinogenic activity of these compounds. Moreover, since these agents are often administered in combination with conventional therapeutic drugs, it is very important to further explore the potential benefits of herb-drug interactions. Previously we have investigated such herb-drug interactions at the level of conjugation.<sup>5,6)</sup> We have also recently established an assay system for conjugation reactions of 1-naphthol using a human adenocarcinoma cell line, Caco-2.<sup>7)</sup> In our current study, to elucidate the possible interaction of both turmeric and curcumin with the conjugation pathways in cells, which in many cases are involved in the activation of procarcinogens, we measured their effects on the conjugation activity of 1-naphthol in Caco-2 cells.

### MATERIALS AND METHODS

**Materials** Powdered turmeric was obtained from Wako, Ltd. and curcumin was purchased from Sigma. Water-soluble and DMSO-soluble turmeric solutions were prepared by stir-

ring turmeric powder (10 g) in either 100 ml distilled water or DMSO for 3 h at room temperature. Each solution was then filtered.

**Cell Culture** Human adenocarcinoma Caco-2 cells were grown in 12-well plates in 1 ml minimal essential medium (MEM) supplemented with 10% fetal bovine serum and additional non-essential amino acids. The cells were seeded at  $5 \times 10^5$  cells/ml and cultured for up to 3 weeks with medium changes every 4–5 d.

**Analyses of 1-Naphthyl Sulfates and Glucuronide** 1-Naphthol (200  $\mu$ M) was added to the culture medium and the cells were further incubated at 37 °C. Aliquots of 50  $\mu$ l of medium were removed at various times, and 30  $\mu$ l of each of these samples was then filtered and injected into a HPLC apparatus, equipped with an ODS column (Chromolith Performance RP-18e, 100 $\times$ 4.6 mm, Merck). The mobile phase consisted of 2 mM tetrabutylammonium hydrogen sulfate in water and acetonitrile (65 : 35). The flow rate was 1.0 ml/min with a column temperature of 40 °C and 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. The effects of curcumin or turmeric on conjugation reactions were thus measured by addition to the culture medium.  $IC_{50}$  values for the concentration-activity curves were calculated using a curve-fit program for Windows.

**RNA Isolation and RT-PCR** Total RNA was isolated from cultured cells *via* the guanidium thiocyanate phenol-chloroform extraction method. First, strand cDNA synthesis and PCR were performed according to the manufacturer's protocol. The PCR primers used to amplify human SULT and UGT cDNAs were as follows: SULT1A1, forward 5'-TTCAAAGCCCCAGGGATTC-3' and reverse 5'-GGCC-ATGTGGTAGAACTG-3'; SULT1A3, forward 5'-GTCA-

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ATGATCCAGGGGAAC-3' and reverse 5'-TTCCATAC-GGTGGAATG: UGT1A1, forward 5'-AACAAGGAG-CTCATGGCCTCC-3' and reverse 5'-GTTCGCAAGATT-CGATGGTCG-3': UGT1A6, forward 5'-ATGGCCTGCCT-CCTTCGCTCA-3' and reverse 5'GGGCATGACCGGCT-AGGATA-37. The cDNA levels were normalized by  $\beta$ -actin, assessed by quantitative real-time PCR as described previously.<sup>6)</sup> Quantitative real-time-PCR was performed with an ABI-Prism 7700 thermal cycler using a SYBR green PCR core reagent kit (Applied Biosystems, Wharmington, U.K.). Calculations of the initial amounts of mRNA were performed according to the cycle threshold method.<sup>8)</sup>

**Assay of SULT and UGT Activities** Phenol SULT (P-SULT) activity within the cytosolic fractions was determined using [<sup>35</sup>S]PAPS as the sulfate donor and 1-naphthol as the sulfate acceptor, according to a slight modification of the procedure of Foldes and Meek.<sup>9)</sup> Briefly, the reaction mixture (250  $\mu$ l) contained 10 mM phosphate buffer (pH 7.4), 50  $\mu$ M 1-naphthol, 5.0  $\mu$ M [<sup>35</sup>S]PAPS (0.4  $\mu$ Ci) and cytosolic extract (50  $\mu$ g of protein). The mixture was incubated at 37 °C for 30 min and the reaction was stopped by the addition of 50  $\mu$ l cold 0.1 M barium acetate. Excess [<sup>35</sup>S]PAPS was precipitated by the addition of 50  $\mu$ l of both 0.1 M Ba(OH)<sub>2</sub> and 0.1 M ZnSO<sub>4</sub>, and removed by centrifugation at 12000 *g* for 5 min. This precipitation procedure was then repeated, and the remaining supernatant (300  $\mu$ l) was transferred to a 3 ml liquid scintillator to determine radioactivity levels. Control reactions were established by omitting the acceptor substrate from the mixture. UGT activity was measured as previously described,<sup>10)</sup> with slight modifications. Briefly, the reaction mixture (50  $\mu$ l) contained 100 mM Tris-HCl (pH 7.4), 10 mM MgCl<sub>2</sub>, 400  $\mu$ M 1-naphthol and a microsomal fraction (100  $\mu$ g of protein). The reactions were initiated by the addition of 0.2 mM UDP-[U-<sup>14</sup>C]glucuronic acid (0.1  $\mu$ Ci) and continued for 30 min at 37 °C. The reactions were stopped by the addition of 100  $\mu$ l ethanol (95%), and the mixtures were then centrifuged at 1000 *g* for 5 min. Aliquots (50  $\mu$ l) were applied to silica-gel TLC plates (Merck 60F254) and developed in *n*-butanol-acetone-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).

## RESULTS

**Effects of Turmeric and Curcumin on Conjugation Reactions in Caco-2 Cells** To elucidate the possible interaction of turmeric and its major constituent curcumin with conjugation reactions in cells, we used the Caco-2 cell line to monitor the glucuronidation and sulfation of 1-naphthol as described previously.<sup>5,6)</sup> After supplementing Caco-2 cultures with 1-naphthol (200  $\mu$ M), the accumulation of 1-naphthyl sulfate and glucuronide in the growth medium was determined by the analytical HPLC over a 24 h period. As shown in Fig. 1, 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). A strong reduction in sulfo-conjugation and a moderate inhibition of glucuronida-

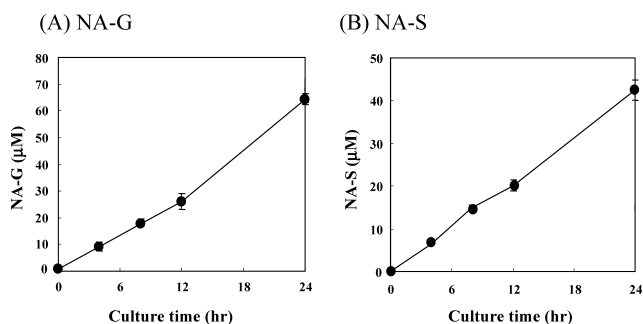


Fig. 1. Accumulation of Glucuronosyl- and Sulfo-Conjugates in the Culture Medium of Caco-2 Cells; (A) Naphthyl Glucuronide (NAG) and (B) Naphthyl Sulfate (NAS)

1-Naphthol (200  $\mu$ M) was added to the culture medium, and the cells were incubated at 37 °C for up to 24 h. Aliquots (50  $\mu$ l) were removed at the various indicated time-points and analyzed by HPLC. Each point represents the mean  $\pm$  S.D. of four assays.

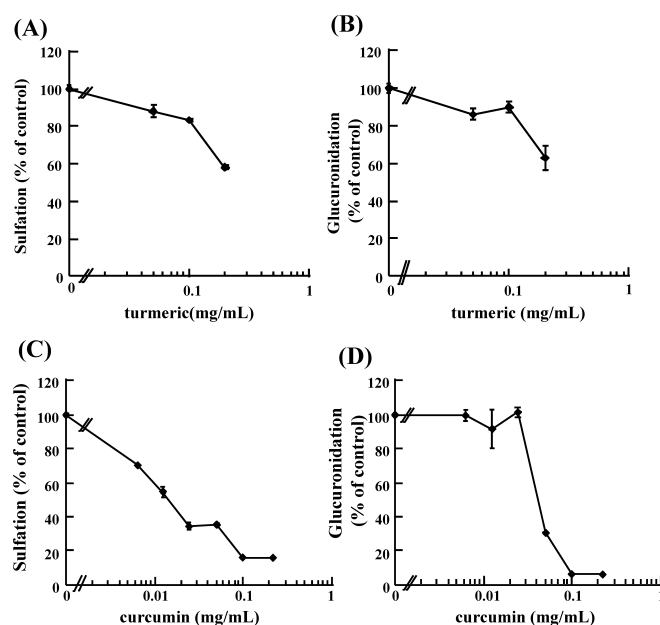


Fig. 2. Effects of Turmeric and Curcumin on Conjugation Reactions in Caco-2 Cells

Caco-2 cells were incubated with 200  $\mu$ M 1-naphthol in the presence or absence (control) of turmeric (A and B) or curcumin (C and D) for 24 h. Conjugate levels (A and C, sulfates; B and D, glucuronide) were determined as described in Fig. 1 and are represented as a percentage of the control. Each value is representative of the mean  $\pm$  S.D. of three experiments.

tion were also observed in the cells treated with DMSO-soluble turmeric (Fig. 2A, B), but no effects were observed with water-soluble materials (data not shown). Because of interference between the turmeric constituents and conjugates in the HPLC chart, the maximum dose of turmeric used was 0.2 mg/ml. IC<sub>50</sub> values were estimated by extrapolating from the data (IC<sub>50</sub>=0.24, 0.29 mg/ml, respectively).

Curcumin, a major anti-oxidant constituent of turmeric, was also found to inhibit sulfo-conjugation in Caco-2 cells at low concentrations (IC<sub>50</sub>=9.7  $\mu$ g/ml, Fig. 2C), but it only showed weak inhibition against glucuronosyl conjugation of 1-naphthol in these cells, except at very high concentrations (IC<sub>50</sub>=40  $\mu$ g/ml, Fig. 2D).

**Effects of Turmeric and Curcumin on *in Vitro* Conjugation Reactions** To further elucidate the mechanisms underlying the inhibitory effects of turmeric and curcumin on

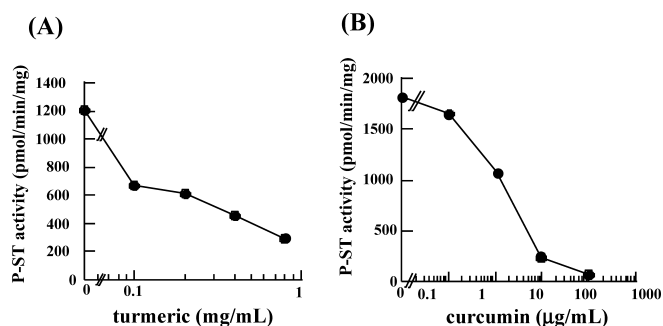


Fig. 3. Effects of Turmeric and Curcumin on the Phenol SULT Activity in Caco-2 Cells

Phenol SULT activity toward 1-naphthol ( $50 \mu\text{M}$ ) in lysates ( $5 \mu\text{g}$  of protein) of Caco-2 cells was assayed in the presence of turmeric (A) or curcumin (B).

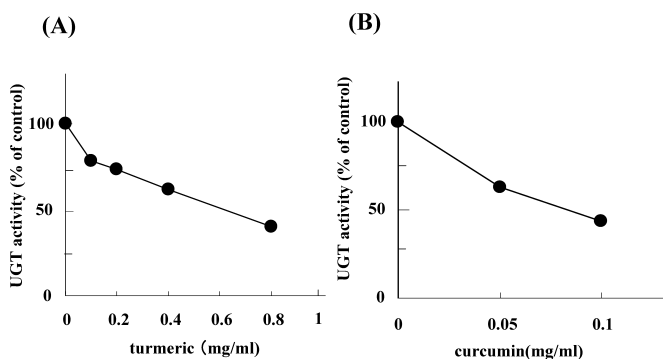


Fig. 4. Effects of Turmeric and Curcumin on the UGT Activity in Caco-2 Cells

UGT activity toward 1-naphthol ( $400 \mu\text{M}$ ) in the microsomes ( $100 \mu\text{g}$  protein) of Caco-2 cells was determined in the presence of turmeric (A) or curcumin (B). Values are normalized as a percentage of the controls. Each value represents the average of two assays. Deviations were within 5% of the values.

1-naphthol conjugation, we next measured the effects of these compounds on the activities of both sulfo- and glucuronosyl conjugation *in vitro*. Turmeric inhibits the phenol sulfotransferase (P-SULT) activity in the cell lysates and UDP-glucuronosyl transferase (UGT) activities in the microsomes of Caco-2 cells ( $\text{IC}_{50}$ =0.21, 0.62 mg/ml, respectively) (Figs. 3, 4). Curcumin also strongly inhibits the P-SULT activity with an  $\text{IC}_{50}$  of  $2.4 \mu\text{g}/\text{ml}$  (Fig. 3B) and exhibits a moderate level of inhibition against the UGT activity ( $\text{IC}_{50}$ =0.079 mg/ml) (Fig. 4B).

**Effects of Turmeric and Curcumin on the Gene Expression of the SULT and UGT Isozymes** There is accumulating evidence that turmeric and curcumin mediate their effects by modulating several important molecular targets, including transcription factors such as NF- $\kappa$ B, AP-1, Egr-1,  $\beta$ -catenin and PPAR $\gamma$ .<sup>2,3</sup> To determine whether turmeric also affects the gene expression of various SULT and UGT conjugating enzymes, we selected SULT1A1, SULT1A3, UGT1A1 and UGT1A6, based upon their phenolic preference. We measured the mRNA levels of these genes in the cells after 24 h of treatment by turmeric using real-time PCR analysis. The results of these experiments showed no significant changes in the expression of P-SULT genes (SULT1A1 and SULT1A3), whereas the induction of UGT1A1 and UGT1A6 could be observed (Fig. 5). In particular, UGT1A1 was significantly induced by 1.7-fold at the highest concentration of turmeric (0.2 mg/ml). Curcumin also induced the

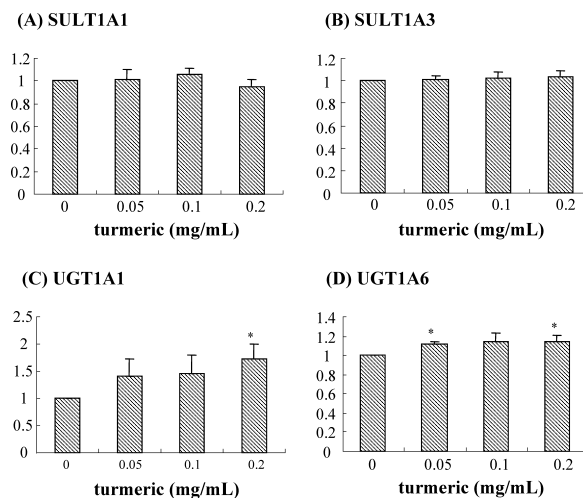


Fig. 5. Effects of Turmeric on the Expression of SULT and UGT Isozyme Genes

The relative amounts of mRNA for both SULT and UGT genes in the cells after 24 h of treatment with turmeric were determined by real-time PCR analysis. Calculations were performed according to the cycle threshold method. (A) SULT1A1, (B) SULT1A3, (C) UGT1A1 and (D) UGT1A6. Each value is an arbitrary unit, with the controls assigned a value of 1.0, that is representative of three experiments ( $* < 0.05$ ).

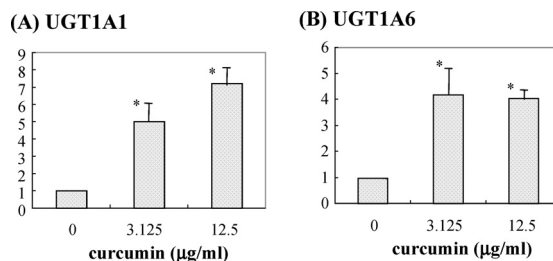


Fig. 6. Effects of Curcumin on the Expression of UGT Isozyme Genes

The relative amounts of mRNA for UGT1A1 and UGT1A6 genes in the cells after 24 h of treatment with curcumin were determined by real-time PCR analysis. Calculations were performed according to the cycle threshold method. (A) UGT1A1 and (B) UGT1A6. Each value is an arbitrary unit, with the controls assigned a value of 1.0, that is representative of three experiments ( $* < 0.05$ ).

gene expression of UGT1A1 and UGT1A6 as shown in Fig. 6. UGT1A1 and UGT1A6 were induced by 7.2-fold and 4.0-fold, respectively, at  $12.5 \mu\text{g}/\text{ml}$  curcumin. No significant effect on SULT genes was observed by curcumin (data not shown).

## DISCUSSION

Our present study has revealed that both turmeric and curcumin inhibit the conjugation of 1-naphthol in the Caco-2 cell line, which is derived from a human colon carcinoma. In particular, both compounds were found to strongly inhibit sulfoconjugation with low  $\text{IC}_{50}$  values ( $0.24 \text{ mg}/\text{ml}$  for turmeric and  $9.7 \mu\text{g}/\text{ml}$  for curcumin). As curcumin comprises 5–10% of most turmeric preparations, the inhibitory effects of turmeric on sulfoconjugation might be mostly attributable to the action of curcumin. On the other hand, although turmeric inhibits the glucuronidation of 1-naphthol to almost the same extent as sulfation ( $\text{IC}_{50}$ =0.29 mg/ml), curcumin shows less inhibitory activity against glucuronidation ( $\text{IC}_{50}$ =40  $\mu\text{g}/\text{ml}$ ). These results suggest that turmeric contains constituents other than curcumin that possess inhibitory

activity toward glucuronidation in Caco-2 cells. Our current data also show that turmeric and curcumin inhibit *in vitro* P-SULT activity at levels that are comparable to those observed in Caco-2 cells. These results therefore suggest that curcumin directly inhibits sulfotransferase activity in the cell.

In many earlier studies, turmeric and curcumin have been shown to have beneficial effects in the treatment of a number of disorders such as cardiovascular diseases, arthritis and cancer.<sup>2,3</sup> Furthermore, as sulfo-conjugation has been shown to be responsible for the bioactivation of proximal carcinogens,<sup>11</sup> the inhibitory activity of turmeric (and curcumin) toward sulfation might underlie their observed anticancer effects. Hence, further characterization of these compounds should be carried out to further clarify their physiological effects against sulfoconjugation.

In this report we have investigated the effects of turmeric and curcumin on the expression of both SULT and UGT genes. Whereas no obvious changes in the levels of SULT gene expression were detectable, significant induction of the UGT1A family of genes, particularly UGT1A1 could be observed (Figs. 5, 6). Recent studies have demonstrated that turmeric and curcumin can affect several drug metabolizing enzymes in mammalian cells by regulating their gene expression.<sup>2,3,12</sup> Induction of different isoforms of the UGT1A subfamily in Caco-2 cells by dietary flavonoids, such as chrysin and quercetin, have also been reported.<sup>13</sup> Although turmeric contains many phenolic compounds with antioxidant properties, curcumin might play an essential role in the induction of UGT1A genes as demonstrated in Fig. 6. High doses (>0.1 mg/ml) of turmeric were found in our current experiments to moderately inhibit the glucuronidation in Caco-2 cells but the induction of UGT1A genes was observed at lower concentration (0.05 mg/ml). This suggests that a lower level of turmeric or curcumin consumption would accelerate UGT1A activity in Caco-2 cells. Since many reports

suggested glucuronidation detoxifies procarcinogens,<sup>12,14</sup> chemoprotective effects may be induced via the induction of UGT enzymes by turmeric and curcumin. Together with the inhibition of sulfoconjugation, turmeric and curcumin may thus modify conjugation reactions in the intestine and the colon, which would modulate the bioavailability of orally administered drugs and affect the toxicity of other chemicals, including (pro)carcinogens and nutrients.

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