UGT1A10 is Responsible for SN-38 Glucuronidation and its Expression in Human Lung Cancers

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Abstract. *Background: We previously reported that up-regulation of glucuronidation activity catalyzed by uridine 5'diphosphoglucuronosyltransferase (UGT) is one of the mechanisms associated with irinotecan hydrochloride/7-ethyl-10-hydroxycamptothecin (CPT-11/SN-38) resistance. In order to extend this result to the clinical setting, it is important to elucidate the role of SN-38 glucuronidation by UGT1A isoforms in CPT-11/SN-38 resistance in vivo. Materials and Methods: We examined SN-38 glucuronidation activity in COS-7 cells transfected with full-length cDNAs for human UGT isoforms (UGT1A1, UGT1A3, UGT1A6 and UGT1A10). The gene expression levels of UGT isoforms were examined in lung cancer cell lines and 14 lung cancer samples by semi-quantitative RT-PCR. Results: Our HPLC assay results showed that both UGT1A1 and UGT1A10 are responsible for SN-38 glucuronidation. The levels of UGT1A1 and UGT1A10 expression in a CPT-11/SN-38-resistant cell line were increased compared to levels in the parent cell line. Furthermore, there was considerable intersubject variability in 14 lung cancer samples, but UGT1A1 and UGT1A10 expression levels were significantly correlated (r=0.70, p=0.004). Our results suggest that not only UGT 1A1, but also UGT 1A10, plays an important role in detoxifying CPT-11/SN-38, leading to resistance to CPT-11/SN-38 in lung cancer.*

Irinotecan hydrochloride (CPT-11) is one of the analogues of camptothecin, an anti-tumor agent isolated from extracts

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Key Words: UGT, CPT-11, SN-38, lung cancer.

of the Chinese tree *Camptotheca acuminate*. CPT-11 has been shown to be a very promising agent against human lung cancers (1, 2). At present, two major metabolic pathways of CPT-11 have been reported. One pathway is bioactivation of CPT-11 by carboxylesterases to the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) (3). SN-38 has been shown to undergo glucuronidation by uridine 5'diphosphoglucuronosyl-transferase (UGT) to form inactive SN-38 glucuronide (4). The other pathway is through oxidation by cytochrome P450. Several oxidative CPT-11 metabolites have been identified in human plasma (5, 6).

Several mechanisms of resistance to CPT-11 have been reported. A point mutation of DNA topoisomerase I gene was described *in vitro* (7). However, we showed that no alteration of DNA topoisomerase I gene was observed *in vivo* and that a low DNA topoisomerase I expression is associated with resistance to CPT-11 (8). It has also been suggested that reduced drug accumulation mediated by the ATP-dependent efflux pump may be involved in a mechanism of resistance to CPT-11 (9, 10).

We previously reported that increased intracellular drug detoxification through the up-regulation of glucuronidation activity catalyzed by UGT is one of the mechanisms in CPT-11/SN-38 resistance in lung cancer cell lines (11). In order to extend our previous study to the clinical setting, it is important to elucidate the *in vivo* role of SN-38 glucuronidation by UGT1A isoforms in CPT-11/SN-38 resistance. In this study we investigated the gene expression levels of UGT1A isoforms, which are responsible for SN-38 glucuronidation, in human lung cancer.

Materials and Methods

CPT-11, SN-38 and SN-38 glucuronide were provided by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan) and Yakult Honsha Co., Ltd. (Tokyo, Japan). CPT-11 and SN-38 were each dissolved in

dimethyl sulfoxide (DMSO) and stored at -80˚C. All other chemicals were commercial products of analytical grade.

COS-7 cells transfected with the full-length cDNA of the human UGT 1A isoforms (UGT1A1, UGT1A3, UGT1A6 and UGT1A10) were as described previously (12). Human lung adenocarcinoma cells, PC-7 and their CPT-11/SN-38-resistant cells, PC-7/CPT, were kindly provided by Dr. Nagahiro Saijo (National Cancer Center Research Institute, Tokyo, Japan). All cells were maintained in RPMI 1640 medium (Nissui Co., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (Mitsubishi Kasei Co., Tokyo Japan), penicillin (100 U/ml) and streptomycin (100 mg/ml) in a humidified chamber (37˚C, 5% CO_2).

Fourteen lung cancer samples from 14 patients (12 males and 2 females; age range 57–86 years, median 68 years) admitted to Hiroshima University Hospital, Japan were studied. Fresh lung cancer samples were obtained during autopsy after informed consent had been obtained. Of these patients, 11 had non-small cell carcinoma and 3 had small cell carcinoma. Eight samples were obtained from patients who had never received chemotherapy while six samples were obtained from patients who were administered platinum drug-containing chemotherapy. The tissues were frozen in liquid nitrogen and stored at -80˚C until analysis.

The SN-38 glucuronidation activities of microsomal preparations from cDNA-transfected COS-7 cells were measured as described previously (11, 12). Briefly, the reaction mixture (200 µl) containing 5 µM SN-38, 5 mM $MgCl_2$, 2mM uridine 5'-diphosphoglucuronic acid, 200 µg microsomal preparations from cDNA-tranfected COS-7 cells and 0.2 M Tris-HCl (pH 7.4) was incubated at 37˚C for the indicated periods. The reaction was terminated by the addition of 1000 µl methanol. After centrifugation at 15000 rpm for 5 min, the supernatant was evaporated to dryness. The SN-38 glucuronide concentrations were determined using a modified high-performance liquid chromatography (HPLC) assay as previously reported (13). Authentic SN-38-glucuronide was used as the standard.

Total cellular RNA extraction and cDNA synthesis were performed as described previously (14). The reverse-transcribed cDNA from each sample was amplified by reverse-transcription polymerase chain reactions (RT-PCR) using specific primers based on UGT1A1, UGT1A10 and β-actin (internal control) gene sequences. We used the UGT1A1 and UGT1A10 primers and PCR conditions as described previously (15). The PCR products were 644 and 478 base pairs long, corresponding to UGT1A1 and UGT1A10, respectively. For a control we used the β-actin gene primers and PCR conditions described previously (14). PCR products were electrophoresed on 2% (w/v) agarose gels, transferred to nylon membranes (Hybond N+; Amersham) and detected by hybridization with ^{32}P-labeled cDNA probes. After washing each filter, the radioactivity level was measured with a laser imaging analyzer (BAS-2000; Fuji Photo Film, Tokyo, Japan). The radioactivity associated with the gene expression level in each sample was expressed relative to that from the β-actin expression level in that sample.

Contingency table analyses based on χ^2 statistics were used to determine the significance of association between categorical variables. All the gene expression levels were skewed towards higher expression levels and were subjected to logarithmic transformation to approximate more closely to a normal distribution. Subsequently Pearson's correlation analysis was performed. The differences at p values of less than 0.05 were considered significant.
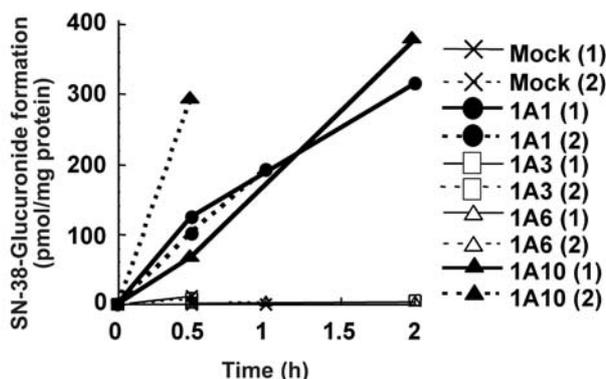

Figure 1. *The reaction mixture containing 5 µM SN-38 and 200 µg microsomal preparations from cDNA-transfected COS-7 cells was incubated at 37˚C. Although we could not determine the exact values of K_m and V_{max}, we detected SN-38 glucuronide formation in both UGT1A1- and UGT1A10-transfected cells. SN-38 glucuronide was not detected in mock-transfected cells or in cells transfected with UGT1A3 or UGT1A6.*

Results

UGT1A isoform activity by HPLC assay. The UGT activities of microsomal preparations from cDNA-transfected COS-7 cells towards SN-38 were determined. After 2 hours of incubation, there were measurable amounts of SN-38 glucuronide not only in the cells transfected with UGT1A1, but also in cells transfected with UGT1A10, with the amount present being time-dependent. In contrast, SN-38 glucuronide was not detected in mock-transfected cells or in cells transfected with UGT1A3 or UGT1A6 (Figure 1).

UGT1A1 and UGT1A10 gene expression. In order to investigate whether the expression of both genes is associated with resistance to CPT-11/SN-38, we examined gene expression in CPT-11/SN-38-resistant human lung cancer cells, in PC-7/CPT cells and in their parent PC-7 cells. We detected UGT1A1 and UGT1A10 gene expression in PC-7/CPT cells but not in PC-7 cells, indicating that the expression levels for both genes increased in CPT-11/SN-38-resistant cells (Figure 2).

We next examined the expression levels of both genes in 14 lung tumor samples obtained from 14 lung cancer patients. There was considerable intersubject variability in the levels of expression of the UGT1A1 and UGT1A10 genes, but within subjects the levels were significantly correlated ($r=0.70$, $p=0.004$, using Pearson's correlation analysis; Figure 3).

Discussion

This study demonstrated that both UGT1A10 and UGT1A1 are responsible for SN-38 glucuronidation. To our knowledge, this is the first study to examine gene expression
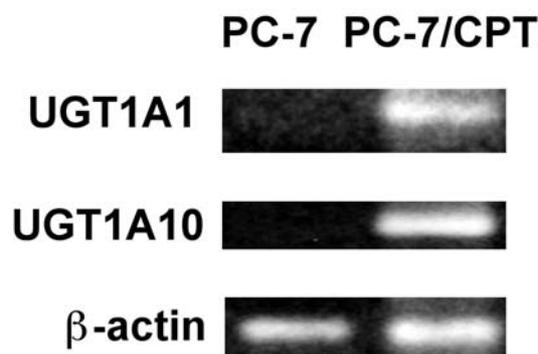
Figure 2. *Expression of UGT1A1 and UGT1A10 genes in PC-7/CPT cells compared with those in PC-7 cells by ethidium bromide staining.*

Figure 3. *The relationship between UGT1A1 and UGT1A10 gene expression in lung cancer samples. Statistical differences were analyzed using Pearson's correlation analysis. A probability value of p<0.05 was considered indicative of significance.*

levels of UGT1A1 and UGT1A10 in lung cancer samples. Our data show that the expressions of UGT1A1 and UGT1A10 genes are significantly correlated.

UGT1A1 was first identified as a main UGT1A isoform involved in SN-38 glucuronidation (4) and our present results confirm this. More recent reports showed that UGT1A7 and UGT1A9 also lead to major components for SN-38 glucuronidation, whereas UGT1A6, UGT1A8 and UGT1A10 play minor roles in SN-38 glucuronidation (16, 17). Although we could not determine the exact values of K_m and V_{max}, we detected SN-38 glucuronide formation in both UGT1A1- and UGT1A10-transfected cells. Furthermore, we did not detect any SN-38 glucuronide formation in UGT1A6 cells. The reason for the discrepancy between our and previous results is unknown, and hence further studies may be required to elucidate the precise role of UGT1A10 in CPT-11/SN-38 metabolism.

The liver and gastrointestinal tract are the major sites of drug metabolism. UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A9 are expressed in hepatic tissue, whereas UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A10 are expressed in biliary tissue (15). In the gastrointestinal tract, UGT1A3, UGT1A6, UGT1A7 and UGT1A10 are expressed in gastric tissue, whereas UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9 and UGT1A10 are in colon tissue (18, 19). The differential distribution of UGT1A loci indicates that the transcriptional regulation of UGT1A isoforms is tissue-specific.

The existence of genetic polymorphisms among members of the UGT1A1 isoforms affects the glucuronidation of SN-38, leading to a protective role of these enzymes in the toxicity of CPT-11 (20); however, polymorphism of UGT1A10 has not been described. Diarrhea is recognized as the main dose-limiting toxicity of CPT-11, being caused by high levels of SN-38 that are retained for a long period in the intestine. SN-38 glucuronide is excreted into the bile and urine and part of it is deconjugated by β-glucuronidase of the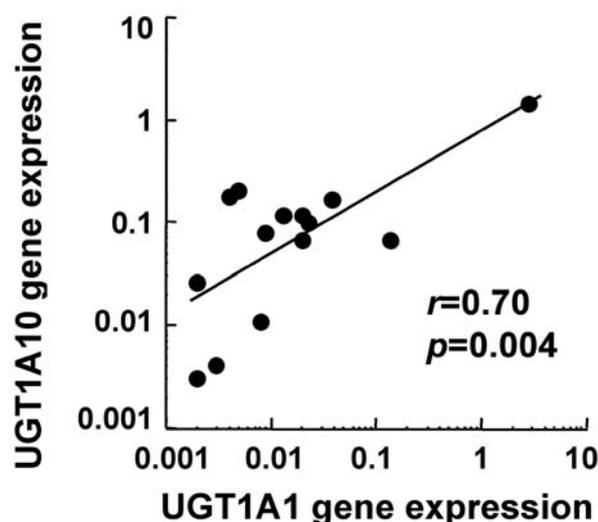 intestinal microflora (21, 22). Considering that UGT1A10 is predominantly expressed in the gastrointestinal tissues, the SN-38 glucuronidation capacity of intestinal UGT1A10 may be another important determinant of CPT-11-related diarrhea.

The intracellular activity of detoxification-related enzymes is associated with drug sensitivity/resistance. We previously demonstrated that up-regulation of glucuronidation activity catalyzed by UGT contributes to resistance to CPT-11/SN-38 (11). These results lead to the hypothesis that expression of UGT1A isoforms involved in SN-38 glucuronidation is a determinant of CPT-11/SN-38 efficacy against cancers. We examined levels of UGT1A1 and UGT1A10 expression in human lung cancer cell lines and found increased expression of both genes in CPT-11/SN-38-resistant cells compared to that of the parent cells. Furthermore, because there are few reports describing the expression levels of UGT1A isoforms in cancers, we examined the expression levels of UGT1A1 and UGT1A10 genes in lung cancers, which revealed considerable intersubject variability and coordinate expression of both UGT1A1 and UGT1A10 genes. These results suggest that the capacity of SN-38 glucuronidation by UGT1A isoforms differs between individual cancers, leading to the resistance to CPT-11. In addition, the up-regulation of drug-efflux pumps is considered another determinant for drug resistance, and SN-38 glucuronides are reported as a substrate for several such pumps (10, 23). We suggest that a combination of increased activity of the efflux pump and/or increased detoxification is required for drug resistance (14), and hence future studies should investigate the combination of increased activity of the SN-38 glucuronide efflux pump and increased detoxification by the UGT1A family in drug resistance.

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