Jpn. J. Cancer Res. 88, 1211–1217, December 1997

The Role of Glucuronidation in 7-Ethyl-10-hydroxycamptothecin Resistance *in vitro*

Toshiaki Takahashi,[1] Yasuhiro Fujiwara,[1,2,5] Michio Yamakido,[1] Osamu Katoh,[3] Hiromitsu Watanabe[3] and Peter I. Mackenzie[4]

[1]Second Department of Internal Medicine, [2]Department of Integrated Medicine, Hiroshima University School of Medicine, [3]Department of Environment and Mutation, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734 and [4]Department of Clinical Pharmacology, Flinders University of South Australia, Bedford Park, SA 5042, Australia

Although glucuronidation catalyzed by uridine 5′-diphosphoglucuronosyltransferase (UGT) is a major pathway of drug inactivation in humans, glucuronidation in malignant cells has received little attention as a cause of anti-cancer drug resistance. In this study, we tried to elucidate the role of SN-38 glucuronidation in the CPT-11-resistant human lung cancer cell line PC-7/CPT. PC-7/CPT cells possessed an increased activity to glucuronidate SN-38 compared to the parent cells, PC-7. Furthermore, sensitivity of PC-7/CPT cells to SN-38 was improved by inhibiting UGT activity. Western and northern blot analyses demonstrated that this increased activity was due to increased levels of UGT protein and mRNA. These results not only imply that upregulation of UGT activity in PC-7/CPT cells may contribute in part to SN-38 resistance, but also illustrate the importance of drug metabolism within malignant cells themselves, as a cause of drug resistance.

Key words: UDP-glucuronosyltransferase — Drug resistance — CPT-11 — SN-38 — Lung cancer

The development of resistance to antitumor agents is one of the main reasons for the failure of cancer chemotherapy. Drug inactivation within tumor cells, such as the conjugation of electrophilic compounds with glutathione, catalyzed by glutathione *S*-transferase, is an important mechanism of drug resistance. Glucuronidation catalyzed by members of the large family of UGTs[1] is another major pathway for the metabolic inactivation of a wide range of xenobiotic and endobiotic substrates.[2,3] Recent studies have demonstrated that elevated UGT activities in tumor cell lines contribute to drug resistance to daunorubicin[4] and mycophenolic acid.[5]

CPT-11, a CPT derivative, is a novel antitumor agent that has been shown to have broad spectrum anti-cancer activity in tumor models and is a very promising new agent against several human malignancies.[6,7] Although the precise metabolic pathway of CPT-11 is still unknown, its antitumor activity arises mainly from its hydrolysis product, SN-38,[8,9] and SN-38 has been shown to undergo glucuronidation to form the inactive SN-38-glucuronide.[10] In addition to ester hydrolysis and glucuronidation, oxidative metabolism, probably mediated by cytochrome P450 3A forms, may also be important in CPT-11 metabolism.[11,12]

Several mechanisms of resistance to CPT and its analogues *in vitro* have been reported: (a) resistance mediated by a point mutation of the *topo I* gene, (b) resistance due to a low topo I expression level, (c) reduction of cellular uptake of CPT, and (d) decreased metabolic activation of CPT-11 to SN-38.[13] Although it has recently been shown that a member of the UGT1A family may be involved in the glucuronidation of SN-38,[14] to our knowledge, there has been no study examining the role of SN-38 glucuronidation in CPT-11 and/or SN-38 drug resistance. Therefore, in the present study, we have tried to elucidate the role of SN-38 glucuronidation by members of the UGT1A family in the CPT-11-resistant human lung cancer cell line PC-7/CPT, which has already been shown to harbor a *topo I* gene point mutation and to have decreased ability to convert CPT-11 to SN-38.[15,16]

[5] To whom correspondence should be addressed.

Abbreviations: UGT, uridine 5′-diphosphoglucuronosyltransferase; SN-38, 7-ethyl-10-hydroxycamptothecin; CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; CPT, camptothecin; SN-38-glucuronide, 7-ethyl-10-hydroxycamptothecin glucuronide; topo I, DNA topoisomerase I; DMSO, dimethyl sulfoxide; UDP-GA, uridine 5′-diphosphoglucuronic acid; HPLC, high-performance liquid chromatography; 4-MU, 4-methylumbelliferone; SDS, sodium dodecyl sulfate; PIPES, piperazine-*N,N′-bis*-[2-ethanesulfonic acid].

The names of UDP glucuronosyltransferase forms are those recommended by a subcommittee of the IUBMB-IUPHAR Nomenclature Committee and supported by participants at the 8th International Workshop on Glucuronidation and the UDP Glucuronosyltransferases held in Iowa City, Iowa, May, 1996.

MATERIALS AND METHODS

Materials CPT-11, SN-38 and SN-38-glucuronide were provided by Daiichi Pharmaceutical Co., Ltd. (Tokyo) and Yakult Honsha Co., Ltd. (Tokyo). CPT-11 and SN-38 were each dissolved in DMSO and the solutions were stored at −80°C. All other chemicals were commercial products of analytical grade.

Cell lines and culture PC-7/CPT and its parental cell line PC-7 were kindly provided by Dr. Nagahiro Saijo (National Cancer Center Research Institute, Tokyo). The cells were propagated in RPMI-1640 (Nikken Biomedical Laboratories, Kyoto) containing 10% heat-inactivated fetal calf serum (Mitsubishi Kasei Co., Ltd., Tokyo), penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37°C in a balanced-air incubator with a humidified atmosphere of 5% CO_2.

Assay of UGT activity The ability of extracts derived from PC-7 or PC-7/CPT cells to convert SN-38 to SN-38-glucuronide was studied according to the method of Yokoi *et al.*[17] To prepare cell extracts, cells were homogenized in 0.2 *M* Tris-HCl (pH 7.4) at 4°C using a Polytron homogenizer (Kinematica AG, Lucerne, Switzerland). The reaction mixture (200 μl) containing 10 μ*M* SN-38, 5 m*M* $MgCl_2$, 2 m*M* UDP-GA, 100 μg of cell extract and 0.2 *M* Tris-HCl (pH 7.4) was incubated at 37°C for the indicated periods. The final concentration of DMSO was 0.5% in the reaction mixture. The reaction was terminated by the addition of 1000 μl of methanol. After centrifugation at 15,000 rpm for 5 min, the supernatant was evaporated to dryness. The SN-38-glucuronide concentrations were determined by a method modified from our previously reported HPLC assay.[18] Briefly, the dried extract was reconstituted with 400 μl of mobile phase solution (acetonitrile–50 m*M* disodium hydrogen phosphate (28 : 72) containing 5 m*M* heptanesulfonate, adjusted to pH 2.0 with orthophosphoric acid). After centrifugation, the supernatant (100 μl) was chromatographed on a C_{18} reversed-phase column with a mobile phase adjusted to pH 3.0, using fluorescence detection (excitation 370 nm; emission 430 nm). Authentic SN-38-glucuronide was used as the standard.

Cytotoxicity assay The cytotoxicities of CPT-11 and SN-38 were measured by means of the regrowth assay, as described previously.[19] Triplicate (PC-7) or five replicate (PC-7/CPT) 10 ml cultures, initially containing 2.5×10^4 cells/ml medium and CPT-11 or SN-38 at various concentrations, with or without 50 μ*M* 4-MU (4-MU itself did not exhibit cytotoxicity against PC-7/CPT cells at this concentration; data not shown), were incubated for 7 days at 37°C in a balanced-air incubator with a humidified atmosphere of 5% CO_2. After incubation, cells were counted under a microscope, and the cell growth ratio of treated to control cultures was calculated. The cytotoxicities were determined as IC_{50} values — the concentrations required for 50% inhibition of cell growth of control cultures during incubation for 7 days.

Western blot analysis We examined UGT protein expression in PC-7 and PC-7/CPT cells using western blot analysis. Approximately 1×10^7 exponentially growing cells were washed twice with cold PBS, and homogenized in 100 μl of lysis buffer (20 m*M* Tris-HCl pH 7.4, 0.1% (w/v) SDS, 1% (w/v) Triton X-100, 1% (w/v) sodium deoxycholate), then the homogenate was centrifuged at 15,000 rpm for 30 min at 4°C to remove debris. The protein concentrations in the cell lysates were determined by use of BCA protein assay reagent (Pierce, Rockford, IL). The lysates were mixed with sample buffer (65 m*M* Tris-HCl pH 6.8, 3% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol), and boiled for 3 min. Samples containing 50 μg total protein were subjected to electrophoresis in 7.5% SDS-polyacrylamide gel, and transferred onto a polyvinylidene difluoride (PVDF) membrane ("Immobilon" PVDF, Millipore Co., Bedford, MA) by semi-dry electroblotting. The membrane was blocked overnight at 4°C in PBS with 0.1% Tween 20 (PBS-T), containing 5% fat-free dried milk. After incubation for 1 h at room temperature in PBS-T containing 0.02% (v/v) anti-UGT polyclonal antibody,[20] the membrane was washed with PBS-T and then incubated for a further hour at room temperature in PBS-T containing 0.033% (v/v) rabbit anti-goat IgG antibody conjugated to horseradish peroxidase (Organon Teknika Co., Durham, NC). After a final wash with PBS-T, the UGT protein on the membrane was visualized with an ECL western blotting kit (Amersham, Buckinghamshire, United Kingdom) according to the manufacturer's protocol.

Northern blot analysis Total cellular RNA was prepared by means of the acid guanidinium thiocyanate-phenol-chloroform extraction method.[21] Total RNA (10 μg) was electrophoresed on 1% agarose-formaldehyde gel and transferred to a nylon filter (Hybond N+; Amersham). The blot was prehybridized in 50% formaldehyde, 0.1 *M* PIPES-NaOH, 0.65 *M* NaCl, 5 m*M* EDTA, 0.1% SDS, 5× Denhardt's solution, and 0.1 mg/ml denatured salmon sperm DNA for 3 h at 42°C. After prehybridization, the blot was incubated overnight in hybridization solution (50% formaldehyde, 0.1 *M* PIPES-NaOH, 0.65 *M* NaCl, 5 m*M* EDTA, 0.1% SDS, 5× Denhardt's solution, 10% dextran sulfate, and 0.1 mg/ml denatured salmon sperm DNA) containing ^{32}P-labeled cDNA probe at 42°C. The blot was washed in 0.1× standard saline-citrate–0.1% SDS and then subjected to autoradiography. The cDNA probes used encoded sequences from regions of the unique first exons of UGT1A1, 1A3, 1A6, and 1A9. The probes for UGT1A1 and 1A6 are specific for these isoforms. However, as

UGT1A3, 1A4 and 1A5 are about 90% identical in sequence, the probe for UGT1A3 also recognizes UGT1A4 and 1A5. As UGT1A9 is also about 90% identical in sequence to UGT1A7, 1A8 and 1A10, the probe for UGT1A9 also recognizes UGT1A7, 1A8 and 1A10 as well. Each of the northern blots was stripped and reprobed for β-actin.

Southern blot analysis Genomic DNA from PC-7 and PC-7/CPT cells was prepared[22] and digested with *Eco*RI and *Hin*dIII. Digested DNA (10 μg) was separated by electrophoresis through a 0.8% agarose gel and transferred to a nylon filter (Hybond N+; Amersham). After prehybridization, the blot was incubated in hybridization solution containing cDNA probes for both UGT1A1 and 1A9. The hybridization procedure was the same as that used above.

RESULTS

UGT activity The UGT activity towards SN-38 in cell extracts of PC-7 and PC-7/CPT was determined (Fig. 1). After 72 h of incubation, the amounts of SN-38-glucuronide formed in extracts of PC-7/CPT and PC-7 were 69.7 and 0.3 pmol/mg protein respectively. This result demonstrated a marked increase in the capacity of PC-7/CPT cells to glucuronidate SN-38. Then, the effect of a non-cytotoxic dose of 4-MU on the formation of SN-38-glucuronide in PC-7/CPT cell extracts was examined. 4-MU was used as a competitive inhibitor of UGT, because this compound has been shown to be a substrate for many UGT forms.[3] As shown in Fig. 1, 4-MU inhibited SN-38-glucuronide formation in PC-7/CPT cell extracts.

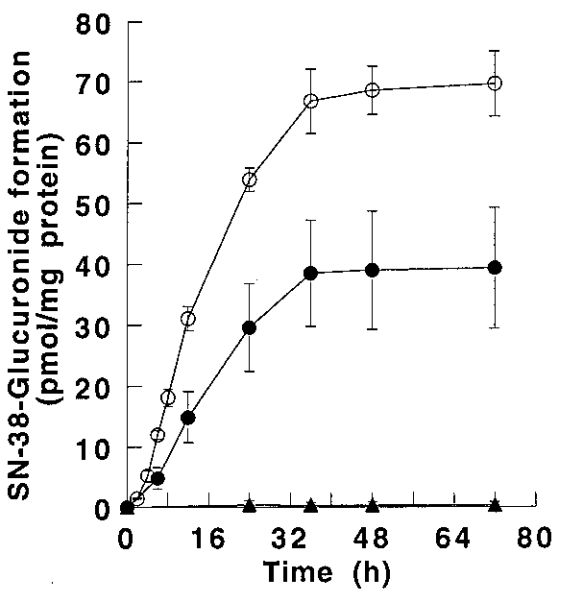

Fig. 1. SN-38-glucuronide formation in extracts of PC-7 (solid triangles) and PC-7/CPT (open circles) cells, and inhibitory effects of 4-methylumbelliferone (4-MU) on SN-38-glucuronide formation in extracts of PC-7/CPT cells (solid circles). To examine the inhibitory effects of a non-cytotoxic dose of 4-MU, 50 μM 4-MU was added to the incubation mixture. Values represent the means±SD of three independent experiments.

Regrowth assay To elucidate whether glucuronidation of SN-38 contributed to the resistance to CPT-11 and SN-38, we tested the effects of the inhibition of UGT activity by 4-MU on the cytotoxicities of CPT-11 and SN-38 against PC-7/CPT cells. Although the sensitivity of PC-7/CPT to CPT-11 was not significantly affected by 50 μM 4-MU, the sensitivity of PC-7/CPT to SN-38 was statistically significantly enhanced by 50 μM 4-MU treatment (Table I). This result suggested that elevated UGT activity in PC-7/CPT cells contributed in part to the resistance to SN-38, but not to CPT-11.

Expression of UGT protein To elucidate whether changes in SN-38-glucuronidation activities corresponded to changes in the amounts of UGT protein, we examined UGT protein expression in PC-7 and PC-7/CPT cells using western blot analysis. As shown in Fig. 2A, a marked increase of UGT protein was observed in PC-7/CPT cells.

Northern blot analysis As marked increases in the activities and protein amounts of UGT were observed in PC-7/CPT cells, the question arose as to whether this reflected increased expression of UGT mRNA. Northern analysis demonstrated that the levels of UGT mRNA recognized with all four cDNA probes were elevated in PC-7/CPT compared with PC-7 (Fig. 2B). The RNA from each of these cell lines appeared to be intact, and equally loaded and transferred, based on ethidium bromide staining of the gel and on hybridization analysis of the membrane with β-actin probe (Fig. 2B).

Table I. Cytotoxicities of CPT-11 and SN-38 to PC-7/CPT Cells

Drug	IC_{50} (μg/ml)	
	4-MU(−)	4-MU(+)
CPT-11	0.36±0.047	0.36±0.025[a]
SN-38	0.0011±0.00011	0.00075±0.000061[b]

Drug cytotoxicity was determined by regrowth assay as described in "Materials and Methods." Each value is the mean ±SD.

a) $P=0.926$ compared to value for 4-MU(−).

b) $P=0.012$ compared to value for 4-MU(−).

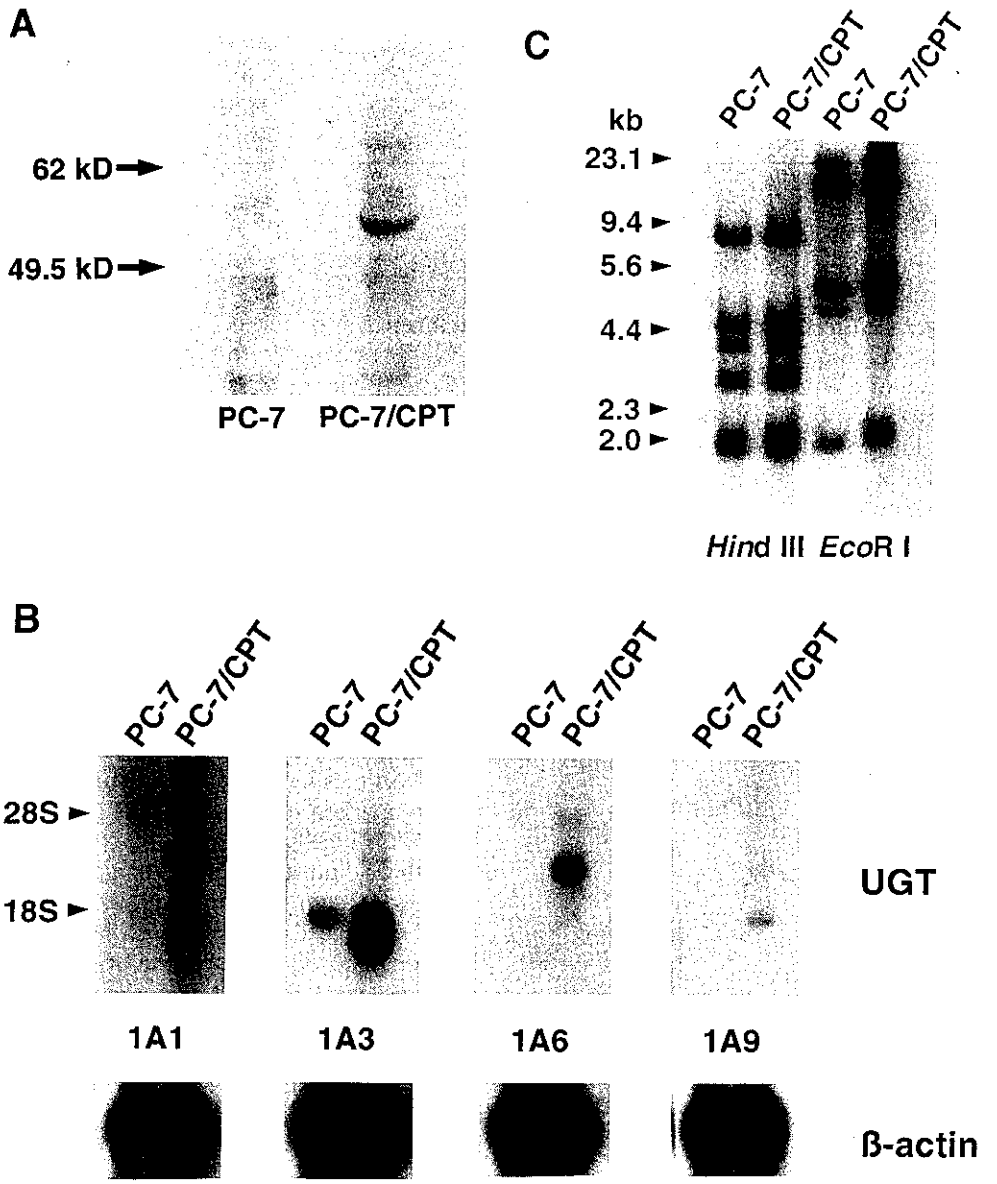

Fig. 2. A, UGT protein expression in PC-7 and PC-7/CPT cells. B, UGT mRNA expression in PC-7 and PC-7/CPT cells. C, Southern blot analysis of the *UGT1A* gene locus from PC-7 and PC-7/CPT cells.

Southern blot analysis To determine whether the increased expression of the UGT1A isoforms in PC-7/CPT cells was due to *UGT1A* gene amplification, Southern blot hybridization analysis was performed. There were no alterations in the size and intensity of the DNA fragments by restriction digestion of PC-7 and PC-7/CPT genomic DNAs (Fig. 2C), suggesting no rearrangement or amplification of the *UGT1A* gene locus in PC-7/CPT cells.

DISCUSSION

In the present study, we found an increase in the capacity to glucuronidate SN-38 due to increased expression of UGT1A isoforms in PC-7/CPT cells, and this may contribute in part to their resistance to the toxic effects of SN-38. Furthermore, this study demonstrates the importance of drug metabolism within malignant cells themselves, as one of the multifactorial mechanisms of anti-cancer drug resistance.

Although glucuronidation is a major pathway for the metabolic inactivation of a wide range of xenobiotic and endobiotic substrates, glucuronidation has received little attention as a possible contributor to anti-cancer drug resistance in malignant cells. The results of the present study clearly showed that CPT-11-resistant PC-7/CPT cells possess elevated UGT activity towards SN-38 compared to the parent cells, PC-7. Furthermore, sensitivity to SN-38 toxicity was improved by inhibiting this UGT activity in PC-7/CPT cells with the UGT substrate, 4-MU.

The elevated capacity of PC-7/CPT cells to glucuronidate SN-38 was correlated with an increase in UGT1A expression as assessed by western and northern analyses. It is conceivable that members of the UGT2B family may also contribute to the elevated levels of SN-38 glucuronidation in PC-7/CPT cells. However, a preliminary investigation with cDNAs expressing UGT2B isoforms has not provided evidence for the participation of this family in SN-38 glucuronidation (unpublished data). Furthermore, a brief report has indicated that the family 1 isoform, UGT1A1 has the capacity to glucuronidate SN-38.[14] Thus, its elevation in PC-7/CPT cells, as shown in this study, may be an important factor contributing to SN-38 resistance.

Ritter *et al.*[23] have suggested that each UGT1A isoform is under the control of its own promoter, thus allowing independent regulation of each isoform at the level of transcription. In this work, we observed that the expression of several UGT1A isoforms was coordinately upregulated in the PC-7/CPT cells. This elevated gene expression was not due to gene amplification or rearrangement of the UGT1A locus, as assessed by Southern analysis. One possible explanation for these results is that the rates of transcription from the *UGT1A* gene locus are much greater in PC-7/CPT cells, for instance, through the activation of a transcriptional enhancer. Further studies are needed to define the mechanisms underlying the elevated *UGT1A* gene expression in these drug-resistant cells.

Although there have been several reports describing multifactorial mechanisms of *in vitro* resistance to CPT and its analogs,[13] the roles of these mechanisms in *in vivo* resistance still remain to be elucidated. *Topo I* gene point mutations have been considered to be one of the main mechanisms of CPT-11 resistance *in vitro*.[13, 16] However, we have recently reported that *topo I* gene mutations may not be the main mechanism, or constitute the initial step, in the development of clinical resistance to CPT-11.[22] In order to elucidate other mechanisms of clinical resistance to CPT-11, we have performed clinical pharmacological evaluations of CPT-11 and SN-38 in patients with small cell lung cancer,[24] but have observed no obvious relationship between clinical pharmacological profiles and response profiles to the drug. In the present study, we have found that glucuronidation of SN-38 is one of the multifactorial mechanisms of resistance to SN-38 toxicity *in vitro*. To our knowledge, there is only one report describing the activity of UGT in human lung cancer *in vivo*,[25] so it is necessary to examine the expression and activities of UGTs in clinically resistant samples in order to extend the present study to the clinical setting.

There are a few reports describing the clinical pharmacokinetics of SN-38-glucuronide in humans.[26–30] In these reports, the investigators found a high degree of inter-individual variability in pharmacokinetic parameters. As inter-individual variability in therapeutic drug response and toxicity is often due to variability in hepatic drug metabolism, and as SN-38-glucuronide is the detoxified metabolite of SN-38, the capacity of individuals to glucuronidate SN-38 may be an important factor determining the response and toxicity after CPT-11 administration. The importance of genetic factors, especially polymorphisms of drug-metabolizing enzymes, as a cause of inter-individual variability of drug effects has been recognized. The UGT isoform, UGT1A1, glucuronidates bilirubin. Recent studies have demonstrated that mutations or polymorphisms in the *UGT1A1* gene or gene promoter result in the inherited unconjugated hyperbilirubinemias of Gilbert's syndrome and Crigler-Najjar syndrome.[31, 32] Although there have been no reports of genetic polymorphisms of other UGT isoforms, population pharmacokinetic studies of CPT-11, SN-38 and SN-38-glucuronide in Caucasians and Japanese[29, 30] suggest ethnic differences in the metabolic profile of CPT-11. For the prevention of adverse reactions and the design of more effective treatment modalities with CPT-11, we are currently identifying specific UGT isoforms responsible for SN-38 glucuronidation and searching for biologically significant genetic polymorphisms in these isoforms.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid from the Ministry of Health and Welfare of Japan, a Japan Society for the Promotion of Science Fellowship for Study at Centers of Excellence Abroad and a grant from Osaka Cancer Research Foundation. We are grateful to Drs. N. Saijo, F. Kanzawa and K. Nishio (Pharmacology Division, National Cancer Center Research Institute, Tokyo) for kindly providing PC-7 and PC-7/CPT cells. We are also grateful to Drs. N. Ohashi (Department of Respiratory Disease, Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital) and T. Isobe, T. Oguri (Second Department of Internal Medicine, Hiroshima University School of Medicine) for their practical suggestions and technical support during the study.

(Received August 15, 1997/Accepted October 2, 1997)

REFERENCES

1) Burchell, B., Nebert, D. W., Nelson, D. R., Bock, K. W., Iyanagi, T., Jansen, P. L. M., Lancet, D., Mulder, G. J., Chowdhury, J. R., Siest, G., Tephly, T. R. and Mackenzie, P. I. The UDP glucuronosyltransferase gene superfamily: suggested nomenclature based on evolutionary divergence. *DNA Cell Biol.*, **10**, 487–494 (1991).
2) Miners, J. O. and Mackenzie, P. I. Drug glucuronidation in humans. *Pharmacol. Ther.*, **51**, 347–369 (1991).
3) Clarke, D. J. and Burchell, B. The uridine diphosphate glucuronosyltransferase multigene family: function and regulation. *In* "Handbook of Experimental Pharmacology," ed. F. C. Kaufman, Vol. 112, pp. 3–43 (1994). Springer Verlag, Heidelberg.
4) Gessner, T., Vaughan, L. A., Beehler, B. C., Bartels, C. J. and Baker, R. M. Elevated pentose cycle and glucuronyltransferase in daunorubicin-resistant P388 cells. *Cancer Res.*, **50**, 3921–3927 (1990).
5) Franklin, T. J., Jacobs, V., Jones, G., Ple, P. and Bruneau, P. Glucuronidation associated with intrinsic resistance to mycophenolic acid in human colorectal carcinoma cells. *Cancer Res.*, **56**, 984–987 (1996).
6) Lavelle, F., Bissery, M. C., Andre, A., Roquet, F. and Riou, J. F. Preclinical evaluation of CPT-11 and its active metabolite SN-38. *Semin. Oncol.*, **23** (Suppl. 3), 11–20 (1996).
7) Dancey, J. and Eisenhauer, E. A. Current perspectives on camptothecins in cancer treatment. *Br. J. Cancer*, **74**, 327–338 (1996).
8) Rivory, L. P., Bowles, M. R., Robert, J. and Pond, S. Conversion of irinotecan (CPT-11) to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by human liver carboxylesterase. *Biochem. Pharmacol.*, **52**, 1103–1111 (1996).
9) Kawato, Y., Aonuma, M., Hirota, Y., Kuga, H. and Sato, K. Intracelluar roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res.*, **51**, 4187–4191 (1991).
10) Atsumi, R., Suzuki, W. and Hakusui, H. Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. *Xenobiotica*, **21**, 1159–1169 (1991).
11) Lokiec, F., Du Sorbier, B. M. and Sanderink, G. J. Irinotecan (CPT-11) metabolism in human bile and urine. *Clin. Cancer Res.*, **2**, 1943–1949 (1996).
12) Haaz, M. C., Rivory, L. P. and Robert, J. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome p-450 3A (CYP3A) and drug interaction. *Proc. Am. Asso. Cancer Res.*, **38**, 17 (1997).
13) Pommier, Y., Tanizawa, A., Okada, K. and Andoh, T. Cellular determinants of sensitivity and resistance to camptothecins. *In* "Camptothecins: New Anticancer Agents," ed. M. Potmesil and H. Pinedo, pp. 123–138 (1995). CRC Press, Boca Raton.
14) Iyer, L., King, C., Tephly, T. and Ratain, M. J. UGT isoform 1.1 (UGT*1.1) glucuronidates SN-38, the active metabolite of irinotecan. *Proc. Am. Soc. Clin. Oncol.*, **16**, 201a (1997).
15) Kanzawa, F., Sugimoto, Y., Minato, K., Kasahara, K., Bungo, M., Nakagawa, K., Fujiwara, Y., Liu, L. F. and Saijo, N. Establishment of a camptothecin analogue (CPT-11)-resistant cell line of human non-small cell lung cancer: characterization and mechanism of resistance. *Cancer Res.*, **50**, 5919–5924 (1990).
16) Kubota, N., Kanzawa, F., Nishio, K., Takeda, Y., Ohmori, T., Fujiwara, Y., Terashima, Y. and Saijo, N. Detection of topoisomerase I gene point mutation in CPT-11 resistant lung cancer cell line. *Biochem. Biophys. Res. Commun.*, **188**, 571–577 (1992).
17) Yokoi, T., Narita, M., Nagai, E., Hagiwara, H., Aburada, M. and Kamataki, T. Inhibition of UDP-glucuronosyltransferase by aglycons of natural glucuronides in *kampo* medicines using SN-38 as a substrate. *Jpn. J. Cancer Res.*, **86**, 985–989 (1995).
18) Sumiyoshi, H., Fujiwara, Y., Ohune, T., Yamaoka, N. and Yamakido, M. High-performance liquid chromatographic determination of irinotecan (CPT-11) and its active metabolite (SN-38) in human plasma. *J. Chromatogr. B*, **670**, 309–316 (1995).
19) Kanzawa, F., Nishio, K., Kubota, N. and Saijo, N. Antitumor activities of a new indolocarbazole substance, NB-506, and establishment of NB-506-resistant cell lines, SBC-3/NB. *Cancer Res.*, **55**, 2806–2813 (1995).
20) Mackenzie, P. I., Gonzalez, F. J. and Owens, I. S. Cloning and characterization of DNA complementary to rat liver UDP-glucuronosyltransferase mRNA. *J. Biol. Chem.*, **259**, 12153–12160 (1984).
21) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159 (1987).
22) Ohashi, N., Fujiwara, Y., Yamaoka, N., Katoh, O., Satow, Y. and Yamakido, M. No alteration in DNA topoisomerase I gene related to CPT-11 resistance in human lung cancer. *Jpn. J. Cancer Res.*, **87**, 1280–1287 (1996).
23) Ritter, J. K., Chen, F., Sheen, Y. Y., Tran, H. M., Kimura, S., Yeatman, M. T. and Owens, I. S. A novel complex locus UGT1 encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes with identical carboxy termini. *J. Biol. Chem.*, **259**, 3257–3261 (1992).
24) Sumiyoshi, H., Fujiwara, Y., Ohashi, N., Egusa, Y., Yamaoka, N. and Yamakido, M. Clinical pharmacological evaluation of CPT-11 and cisplatin (CDDP) in patients with small cell lung cancer (SCLC). *Proc. Am. Soc. Clin. Oncol.*, **14**, 457 (1995).
25) Toussaint, C., Albin, N., Massaad, L., Grunenwald, D., Parise, O., Jr., Morizet, J., Gouyette, A. and Chabot, G. G. Main drug- and carcinogen-metabolizing enzyme

systems in human non-small cell lung cancer and peritumoral tissues. *Cancer Res.*, **53**, 4608–4612 (1993).
26) Gupta, E., Lestingi, T. M., Mick, R., Ramirez, J., Vokes, E. E. and Ratain, M. J. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res.*, **54**, 3723–3725 (1994).
27) Rivory, L. P. and Robert, J. Identification and kinetics of a β-glucuronide metabolite of SN-38 in human plasma after administration of the camptothecin derivative irinotecan. *Cancer Chemother. Pharmacol.*, **36**, 176–179 (1995).
28) Gupta, E., Mick, R., Ramirez, J., Wang, X., Lestingi, T. M., Vokes, E. E. and Ratain, M. J. Pharmacokinetic and pharmacodynamic evaluation of the topoisomerase inhibitor irinotecan in cancer patients. *J. Clin. Oncol.*, **15**, 1502–1510 (1997).
29) Sridhara, R., Fujiwara, Y., Egorin, M. J. and Chabot, G. G. Population pharmacokinetics (PK) in two treatment schedules of irinotecan (CPT-11) and metabolite (SN-38). *Proc. Am. Soc. Clin. Oncol.*, **16**, 206a (1997).
30) Fujiwara, Y., Egorin, M. J., Reyna, S., Tait, N., van Echo, D. A., Yamakido, M. and Sridhara, R. Population pharmacokinetics (PK) of irinotecan (CPT-11) and metabolites. *Proc. Am. Soc. Clin. Oncol.*, **16**, 237a (1997).
31) Jansen, P. L. Genetic diseases of bilirubin metabolism: the inherited unconjugated hyperbilirubinemias. *J. Hepatol.*, **25**, 398–404 (1996).
32) Monaghan, G., Ryan, M., Seddon, R., Hume, R. and Burchell, B. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet*, **347**, 578–581 (1996).