Glucuronidation as a Mechanism of Intrinsic Drug Resistance in Human Colon Cancer: Reversal of Resistance by Food Additives

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ABSTRACT

Colon cancer exhibits inherent insensitivity to chemotherapy by mechanisms that are poorly characterized. We have shown that human colon cancer cells are efficient in drug conjugation catalyzed by UDP-glucuronosyltransferases (UGTs) and now report on the role of glucuronidation in de novo resistance to two topoisomerase I inhibitors. Identification of the UGT responsible for glucuronidation of SN-38 and the anthraquinone NU/ICRF 505 was achieved by first using a panel of human cDNA-expressed isozymes to measure conjugating activity. HT29 colon cancer cells were then probed by reverse transcriptase-PCR, Western Blot analysis, and liquid chromatography with mass spectrometry for their profile and activity of UGT isozymes and screened for effective inhibitors of glucuronidation. Expression analysis was also conducted in colon cancer biopsies and paired adjacent normal colon specimens. UGTIA9 was identified as the isozyme catalyzing biotransformation of the two compounds in HT29 cells and propofol as an effective competitive inhibitor of this metabolism. Inhibition of glucuronidation resulted in up to a 5-fold enhancement in drug activity. The majority of colon cancer biopsies studies expressed UGT protein at levels greater than in HT29 cells but with marked interpatient variations and proficiently glucuronidated the two anticancer drugs. A range of UGT aglycones were capable of modulating glucuronidation in the biopsies with octylgallate being 10-fold more potent (ID_{50} 24 \mu M) than propofol. In a subset of tumors (33%), UGT protein levels and activity exceeded that of paired normal colon. Glucuronidation may represent a mechanism of intrinsic drug resistance in colon cancer open to modulation by a range of food additives and proprietary medicines.

INTRODUCTION

Human CRC ranks as the second leading cause of cancer-related death in Western society (1, 2). Although primary surgery can achieve a cure rate of 50%, survival of patients not cured by surgery continues to be disappointingly low because of poor responsiveness to drug treatment (3). The chemorefractory nature of CRC has implied the underlying mechanisms remains poorly understood (4, 5).

5-FU-based regimens have provided the mainstream for drug treatment in CRC (2, 3, 6). The new drug irinotecan (CPT-11, CAMPTO) offers the potential of a major development in the treatment of this disease, having demonstrated significant clinical activity in 5-FU resistant patients (7), and more recent clinical trials have now established irinotecan in combination with 5-FU as standard first line therapy for disseminated CRC (8–10). Based on the structure of the naturally occurring plant product CPT, irinotecan works by inhibition of the key nuclear enzyme Topo I (11). Irinotecan is a produg for release by carboxylesterases of the potent Topo I inhibitor SN-38 (7). Increasingly, non-CPT-based compounds are being identified as Topo I inhibitors with the promise of distinct pharmacological properties (12, 13). NU/ICRF 505 is an N-tyrosine conjugate of anthraquinone conjugated at the free COOH terminal of the amino acid as an ethyl ester, and although it appears to act as a classic inhibitor of Topo I (14), it is also noncross resistant in CPT-resistant cell lines (15).

MATERIALS AND METHODS

Materials. Standards of the two glucuronide metabolites of NU/ICRF 505 (the ring C4-O-glucuronide and tyrosine glucuronide) and the single glucuronide metabolite of SN-38 (the C10-O-glucuronide) were biosynthesized by incubation with HT29 cells, then purified by column chromatography and finally authenticated as pure by LC/MS (21). NU/ICRF 505/M, the product of hydrolysis of the COOH-terminal ethyl ester linkage yielding the free amino acid, was synthesized, purified, and chemically characterized as described previously (22). SN-38 lactone was from Rhone-Poulenc Rorer (Vitré, France), and SN-38 hydroxy acid was prepared by alkaline hydrolysis of the parent compound (23). All common UGT substrates (aglycones) used were of the highest grade available commercially and were either from Aldrich or Sigma Chemical Co. (Poole, United Kingdom).

Cell Lines. HT29, HCT15, Colo320, LoVo, SW480, H11002, HCT116, and HRT18 human CRC cell lines were obtained from either American Type Culture Collection (Manassas, VA) or European Collection of Animal Cell Cultures (Salisbury, United Kingdom). V79 Chinese hamster fibroblasts and HEK293 fibroblasts stably expressing full-length human UGT cDNAs were as described previously (24, 25). Micromeres prepared from SF-9 insect cells transfected with human UGT1A7 and 1A10 were purchased from Oxford Biomedical Research (Oxford, M1). Control micromeres prepared from SF-9 insect cells infected with wild-type baculovirus were also from Oxford Biomedical Research. All experiments were performed on cells within 10 passages of each other.

Clinical Specimens. Twenty human colon cancer biopsies and 10 paired adjacent normal colon specimens (0.2–0.5 grams) were kindly obtained from Professor Malcolm Dunlop, Western General Hospital (Edinburgh, United Kingdom). Human liver and ovarian cancer specimens were from in house tissue banks. After resection, all colon specimens were immediately snap frozen in solid carbon dioxide and were then stored at –80°C until used in the present study. Written informed patient consent and local committee ethical approval were both obtained to conduct the experimental investigations described in this article.
UGT Isozyme Profiles in Cell Lines. Glucuronidation activity profiles in HT29 and HCT116 cells toward a panel of UGT isoform-specific aglycones using 500 μM substrate and 2 mM [14C]-UDP-glucuronic acid as cofactor were determined by HPLC with quantitative radio-hemiacetal detection (26). The activity of individual UGT isozymes was evaluated in transfected cell lines by LC/MS at low and high (saturating) concentrations of NU/ICRF 505 (10 or 150 μM) and at a saturating concentration of 250 μM for SN-38 (27). Accurate quantitation of the newly formed glucuronides was achieved by calibrating with authentic standards. UGT activity in the transfectants was independently confirmed and measured with isof orm-specific aglycones. Cell lysis was as previously described, and these studies were performed with two to four replicates (26).

Western Blot Analysis of UGT. Human liver (SF9) fraction, cell lysates or homogenized clinical specimens (20 μg protein/well in all cases) were resolved on 10% SDS polyacrylamide gels essentially as described by Laemmli (28). The clinical specimens were lysed by mechanical homogenization at 4°C in 1 ml of a lysis buffer [50 mM Tris (pH 7.5), 1 mM EDTA, 150 mM NaCl, 2 mM sodium orthovanadate, 50 mM sodium fluoride, 20 μM phenylarsine oxide, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin] using a laboratory mixer emulsifier (Silverson Ltd., Chesham, United Kingdom). Proteins were transferred to Immobilon-P membranes (Millipore, Watford, United Kingdom; Ref. 29), blocked, and then incubated with the primary antibody (30). After washing, membranes were incubated with the secondary antibody (ImmuNoPure rabbit antish IgG, H + L) peroxidase conjugated; Pierce, Etten-Leur, The Netherlands). Finally, protein bands were visualized using the chemiluminescence reagent Plus (NEF Life Science Products, Inc., Boston, MA) and quantitated by image analysis using a UVP EpiChemi (3) Bioimaging System (UVP, Inc., Upland, CA).

RT-PCR Analysis of the UGT1A Locus. Total RNA was extracted from HT29 and HCT116 cells using the Tri Reagent technique (Sigma Chemical Co., including DNAse treatment at 37°C) (31). The sense probes for the unique exons 1 and 2 (UGT1A1, IA3-IA10), and antisense primer for a region in the common exon 5 of the UGT 1A locus, 5'-GGTCCGCAAGTGTCATGGTGTCG-3', were as reported previously (32). A common UGT1A sense primer was also incorporated, 5'-TCCAAATTTGTGCAAAACACAGC-3' (32).
cDNA was synthesized using the First Strand cDNA synthesis kit (Boehringer, Mannheim, Germany). RT-PCR reactions were performed under standard conditions using Taq Polymerase (ICRF, Clare Hall, United Kingdom). Reactions were initially incubated for 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, followed by 30 s incubation at the annealing temperature of each primer pair and finally 5 min incubation at 72°C. The annealing temperature values for primer pairs were: 59°C for UGT1A1, IA7 and IA8, and 61°C for UGT1A1-IA6 and 62°C for UGT1A9 and IA10.

NU/IRCF 505 Cellular Accumulation in the Presence of UGT Aglycones. Drug transport studies in HT29 and HCT116 colon cancer cell lines were conducted with NU/ICRF 505 and SN-38 in the presence of the following UGT aglycones: propofol; ibuprofen; methyl 4-hydroxybenzoate; and R- and S-citronellol (23, 33). These studies were performed in 1.25-cm² tissue culture flasks and were repeated in triplicate on at least two separate occasions (Falcon Plastics; Becton Dickinson Labware, Franklin Lakes, NJ). Cells were plated at a density of 10⁶ cells in 4 ml of RPMI media and allowed 24 h to attach before addition of the Topo I inhibitor and UGT aglycone. Drugs were added in 4 ml of media (final DMSO concentration <0.5%) at a final concentration of 5 and 10 μM for NU/ICRF 505 and 1 μM for SN-38 in the presence of 0, 50, 100, and 200 μM of the different UGT aglycones. At 6 and 24 h, cells and tissue culture media samples were collected for quantitative determination of the two Topo I inhibitors, their glucuronide metabolites, hydrolys products, and in the case of the hydroxypropofol, by reversed-phase HPLC with solid-phase extraction (23, 27). Separate 1.25-cm² flasks were used to count cells after the various combinations of drugs, and intracellular drug concentrations were normalized to 10⁶ cells. All drugs were made up fresh in DMSO.

LC/MS Detection of Propofol Glucuronide. An alternative reaction monitoring technique was used for the detection of propofol glucuronide (if formed) in the tissue culture medium of coincubations of NU/ICRF 505 and propofol. A standard of propofol glucuronide was biosynthesized for assay calibration and quantitative determination by incubation with UGT1A9 expressed cell preparations in the presence of UDP-glucuronic acid (26). The analytical system comprised of an HPLC tandem MS (Quattro LC; Micromass, Altrincham, United Kingdom) with electrospray ionization operating in the negative ion mode. The primary acquisition conditions of the electrospray source were capillary voltage, 3.5 kV, cone voltage, 30 V, source block temperature, 100°C; source isolation temperature, 200°C; using nitrogen as the desolvation gas and nebuliser flow (100 and 600 liters/min, respectively). HPLC conditions comprised a binary mobile phase with buffer A as 10 mM ammonium acetate and solvent B acetonitrile with gradient elution from 0–100% B over 8 min on a 25-cm Spherisorb ODS2 column (Waters, Watford, United Kingdom) at a total flow rate of 1 ml/min. The eluant from the HPLC was passed through a 1.5-T-piece splitter, resulting in a flow rate of 200μl/min being delivered to the electrospray source. The multiple reaction monitoring method monitored the transition from 353.3 to 176.8, and the collision energy was optimized to 30 eV.

Media (100 μl) from cells exposed to NU/ICRF 505 and varying concentrations of propofol as described above were treated with 100 μl of prechilled acetonitrile to precipitate proteins by centrifugation at 1000 × g for 10 min. Fifty μl of the supernatant were injected into the LC/MS.

Growth Inhibitory Activity of SN-38 and NU/ICRF 505 in Colon Cancer Cells in the Presence of Propofol. All cell lines were cultured in RPMI 1640 supplemented with 5% heat-inactivated FCS containing a 1% antibiotic mixture under standard conditions and maintained at 37°C in a humidified atmosphere of 5% CO₂ in air.

Log-phase cells (HT29 and HCT116) were exposed to a range of SN-38 (0 and 1–32 μM) and NU/ICRF 505 (0 and 1–40 μM) concentrations for 24 h in the presence of 0, 50, 100, or 200 μM propofol. After treatment, drug-containing media was removed and replaced with fresh nondrug containing media. In the combinations containing propofol, separate control plates containing 50, 100, or 200 μM propofol alone in the absence of a Topo I inhibitor were included as the controls. Cells were cultured for an additional 2–3 days before cell number was established by counting using a particle analyzer (Model ZM; Coulter Electronics, Luton, United Kingdom). All drugs were made up fresh as stock solutions dissolved in DMSO, and the final concentration of DMSO used in all of the different combinations did not exceed 0.5%. Growth inhibition studies were performed in either 6- or 24-well plastic tissue culture plates with four to six replicates/drug combination and were repeated on two to four separate occasions. Cell numbers in the treatment groups were normalized against the appropriate control group and IC₅₀ values derived by linear regression.

In a series of preliminary experiments, growth curves were performed to determine the level of toxicity of propofol in the absence of SN-38 and NU/ICRF 505 before the combination studies. HT29 and HCT116 cells were exposed for 24 h to 0, 50, or 200 μM propofol and cell number determined each day by Coulter counting for up to 4 days after drug exposure. Growth curves were derived from quadruplicate cell counts.

Measurement of SN-38 and NU/ICRF 505 Glucuronide in a Panel of Human Colon Cancer Cell Lines. The eight cell lines were seeded at a density of 10⁵ cells in 4 ml of RPMI media supplemented with 5% heat-inactivated FCS containing a 1% antibiotic mixture in separate 12.5-cm² tissue culture flasks and allowed 24 h to attach before addition of the Topo I inhibitor. Drug was added in 4 ml of media at a final concentration of 10 μM for NU/ICRF 505 or 1 μM for SN-38 and incubated over a period of 24 h. At the end of the incubation, 1 ml samples of culture media were collected for quantitative determination of glucuronide metabolites by reversed-phase HPLC with solid-phase extraction (23, 27). The cells were counted, and glucuronidation activity was then expressed for each cell line as ng glucuronide formed/24 h/10⁶ cells. Duplicate samples of media were analyzed, and incubations were repeated on two separate occasions.

Measurement of SN-38 and NU/ICRF 505 Glucuronidation in Clinical Specimens. Partially thawed clinical specimens for glucuronidation assays were lysed by hand homogenization in prechilled 0.25 M sucrose/5 mM HEPES buffer (pH 7.4). UGT activity was measured by incubating lysates with 150 μM NU/ICRF 505 or 250 μM SN-38 in the presence of 0, 20, 50 100 150, 250, and 500 μM of the following UGT aglycones: propofol; octylgallate; ethinylestradiol; and ibuprofen for 2 h at 37°C. Assays comprised 100 μM Tris Maleate buffer (pH 7.4), 5 mM MgCl₂, 0.1–0.2 mg of protein, and 4 mM UDP-glucuronic acid in a total volume of 40 μl, and activity was measured by LC/MS optimized for direct determination of the glucuronidate metabolites (27).
RESULTS

Identification of the UGT Isozyme(s) Responsible for Catalyzing Topo I Inhibitor Glucuronidation in HT29 Colon Cancer Cells. Initially, a panel of stable transfectants expressing individual UGT isozymes was screened for the ability to accept the two Topo I compounds as substrates (Fig. 1A). SN-38 was most efficiently metabolized by UGT1A1 followed by UGT1A9 but at a 4-fold lower level of activity, then UGT1A7 and finally UGT1A10, at 7% of the UGT1A1 rate, broadly in keeping with a series of recent investigations using similar model systems and analytical techniques (34–36). NU/ICRF 505 was metabolized predominately by UGT1A9, 1A1, 2B7, and to a lesser extent 1A10. The active isozymes displayed a characteristic preference toward the two possible metabolites of NU/ICRF 505 where UGT1A1 generated both in similar proportion, whereas UGT1A9 showed selectivity for the tyrosine glucuronide and UGT1A10 showed selectivity for the ring C-4 glucuronide. Interestingly, UGT2B7 exhibited high activity but with complete specificity for the ring C-4 glucuronide of NU/ICRF 505.

HT29 cells were then probed with isoform selective substrates to identify the spectrum of enzymatic activities present (Fig. 1B). Greatest activity was recorded with 1-naphthol, a UGT1A6 substrate, whereas minimal metabolism was attributable to UGT2B4, UGT2B7, and UGT2B15. High activity was also recorded with the UGT1A9 substrates propofol and octylgallate (37). However, no activity was detected with the UGT1A1 substrate bilirubin.

Because UGT1A6 does not accept either SN-38 or NU/ICRF 505 as a substrate whereas UGT1A1 activity was not present in HT29 cells and UGT1A7 is neither expressed in the colon (16, 17) nor proficient at metabolizing either compound, it follows that UGT1A9 is the most probable isoform responsible for the major amount of SN-38 and NU/ICRF 505 in HT29 cells. However, a contribution from UGT2B7 cannot be excluded in the formation of the C4-glucuronide of NU/ICRF 505. No demonstrable glucuronidation was recorded in HCT116 cells with any of the probe substrates investigated.

RT-PCR was used to identify transcripts for each individual member of the UGT1A locus (Fig. 2). All nine possible transcripts were detected in HT29 cells: UGT1A1 and IA3-IA10, whereas little evidence of any transcript was apparent in HCT116 (Fig. 2). Presence of UGT protein in HT29 cells and almost complete absence in HCT116 were confirmed by Western blot analysis (see inset to Fig. 5).

Propofol Selectively Inhibits Glucuronidation and Enhances the Activity of SN-38 and NU/ICRF 505 in HT29 Cells. After a preliminary screening of a number of different UGT aglycones, the UGT1A9-selective substrate propofol (37) was identified, which inhibited SN-38 glucuronidation in HT29 cells in a concentration-dependent manner (50–200 μM) by up to 91.6%, with the net effect of increasing the concentration of biologically active SN-38 lactone in both the culture media (from 81.5 ± 5.0 to 100.5 ± 5.5 ng/ml, P = 0.01, Student two-tailed t test) and cells (Fig. 3, A and B). Likewise, propofol also inhibited NU/ICRF 505 glucuronidation in a concentration-dependent manner in HT29 cells at a maximum effect of 90.1%, resulting in a 3-fold fall in glucuronide levels detected in culture media and a 10-fold increase in the parent drug from a low level of 0.035 ± 0.015 μg/ml (Fig. 3C). This was accompanied by a 32-fold restoration in intracellular concentrations from 5.4 ± 0.3 ng/10⁶ cells in the absence of propofol to 175 ± 32 ng/10⁶ cells after cotreatment with 200 μM propofol (Fig. 3D). By contrast, propofol had no significant effect on the intracellular and extracellular concentrations of both drugs in HCT116 cells, which were maintained at levels ~2-fold higher for SN-38 and 100–500-fold higher for NU/ICRF 505 compared with HT29 cells (data not shown).

In preliminary experiments, the influence of a 24-h incubation of propofol alone on the growth of HT29 and HCT116 cells was investigated (data not shown). Here, propofol was demonstrated to have no significant effect on HT29 cells over the 4-day incubation period both at 50 and 200 μM, and whereas 50 μM was also without effect on HCT116 cells, 200 μM resulted in a 20% reduction in counts on days 2–4. As a result, a 24-h incubation of propofol alone was chosen as the treatment condition for experiments on HT29 and HCT116 cells. The results showed that propofol inhibited the growth of HT29 but not HCT116 cells (Fig. 3E).
Propofol is Selectively Metabolized to a Glucuronide in HT29 Cells. HPLC analysis of the culture media from HCT116 and HT29 cells detected propofol at concentrations very close to their expected HPLC analysis of the culture media from HCT116 and HT29. SN-38 in media (A) and SN-38 in cells (B) where SN-38 lactone is (○), SN-38 hydroxy acid is (●), and SN-38 glucuronide is (□). NU/ICRF 505 in media (C) and NU/ICRF 505 in cells (D) where NU/ICRF 505 is (○), the C4-glucuronide is (□), and tyrosine glucuronide is (●). Each point represent the mean ± SD of n = 3.

Table 1 Effect of propofol on the IC50 concentration of NU/ICRF 505 and SN-38 in HT29 and HCT116 human colon cancer cells.

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<th>NU/ICRF 505 (μM)</th>
<th>SN-38 (nm)</th>
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<tr>
<td></td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>HT29</td>
<td>17.2 ± 2.62e</td>
<td>12.7 ± 0.85e</td>
</tr>
<tr>
<td>HCT116</td>
<td>7.06 ± 2.15</td>
<td>7.73 ± 1.05</td>
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*Log-phase cells (HT29 and HCT116) were exposed to a range of SN-38 (0 and 1–32 nM) and NU/ICRF 505 (0 and 1–40 μM) concentrations for 24 h in the presence of increasing concentrations of propofol. After a change in media, cells were cultured for an additional 2–3 days before cell number was established by counting using a particle analyser. Cell numbers in the treatment groups were normalized against the appropriate control group, and growth inhibition curves were then constructed. The IC50 for growth inhibition was derived by linear regression analysis.

* Each IC50 represents the mean ± SD from two to four separate experiments.

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of cases, UGT protein expression in the normal colon surpassed that of the paired tumor biopsy (Fig. 6C).

**Glucuronidation Activity in Human Colon Cancer Biopsies Can Exceed That of the Liver and Normal Colon by 5-Fold.** Human colon cancer biopsies metabolized NU/ICRF 505 and SN-38 in a manner analogous to HT29 cells to a degree that followed closely the level of protein expression determined using the RAL antibody (Fig. 7A–C, compare to Fig. 6C). In patient 84, drug conjugation in the colon cancer specimen consistently exceeded that of the paired normal colons by up to 5-fold; in patients 95, 99, and 279, comparable glucuronidation of the two compounds occurred in both tumor and adjacent normal colons, whereas in the remaining 4 paired specimens, UGT activity in the normal colon exceeded that of the paired tumors by up to 10-fold.

**Food Additives and Proprietary Medicines also Inhibit Glucuronidation in Human Colon Cancer Biopsies.** Propofol was able to effectively inhibit glucuronidation in 75% of the colon cancer biopsies studied with a concentration for ID$_{50}$ similar to that observed in HT29 cells (ID$_{50}$ 172–338 μM compared with 143 μM for HT29 cells). Moreover, UGT aglycones were identified that were up to 10 times more potent than propofol, i.e., the food additive octylgallate (ID$_{50}$ 24 μM).

**DISCUSSION**

In this article, evidence is presented that glucuronidation can function as a mechanism of de novo drug resistance to two different Topo I inhibitors, SN-38 and the anthraquinone NU/ICRF 505, in nonselected human colon cancer cells in vitro, accounting for up to 5-fold resistance. Participation of UGTs in acquired resistance to SN-38 has been implied previously in a lung cancer cell line (PC-7/CPT) selected by continuous exposure to irinotecan where coordinate up-regulation of several UGT isozymes (UGT1A1, UGT1A3, and UGT1A6) was shown (38). Nonetheless, the unequivocal involvement of the drug metabolizing enzyme was not demonstrated in the absence of direct detection of the SN-38 glucuronide, whereas evidence was presented of decreased expression of Topo I protein and reduced bioactivation.
of irinotecan to SN-38, alternative mechanisms that could plausibly explain the high level of resistance observed (39). High endogenous glucuronidation activity toward a number of anticancer drugs, including SN-38 and epirubicin, has been observed in human S1 colon cells, leading to speculation of a role for the UGT1A family in intrinsic drug resistance (40). Although, in acquired resistance to SN-38, selected by exposure to mitoxantrone, there was a 2-fold reduction in the activity and expression of the UGT1A family (40). Glucuronidation has also been strongly implicated in intrinsic resistance to the antitumor, immunosuppressive antibiotic mycophenolic acid in HT29 cells (41, 42). In a recent genome-wide cDNA microarray analysis attempting to correlate expression profiles to chemosensitivity in 85 different xenografts, UGT1A1 was 1 of only 32 genes that consistently corresponded to intrinsic drug resistance to a panel of seven different anticancer drugs (43).

Through a combination of studying substrate specificity of expressed UGT isoforms and probing cells with selective UGT substrates and inhibitors, UGT1A9 was strongly implicated as the isozyme accounting for the majority of SN-38 and NU/ICRF 505 glucuronidation in HT29 cells. UGT1A9 has the highest affinity of any known UGT isoform for SN-38 as a substrate when expressed in human HEK cells and is also one of the most catalytically efficient in metabolizing the drug (35). A functional approach was adopted to identify UGT isozymes because it avoided the issue of the numerous polymorphisms that have been described within the UGT1A locus that can result in the expression of proteins with reduced or no catalytic activity (36, 44, 45) that may compromise the interpretation of data generated using genetic-based methodologies. Analysis of function was also necessary in the absence of isoform selective antibodies, which have proved difficult to produce because of close sequence homology between many different members of the superfamily (46).

Coadministration of the UGT1A9 substrate propofol (37) achieved an almost complete abolition of metabolic conversion of both NU/ICRF 505 and SN-38 to their respective glucuronides selectively in HT29 cells and a restoration of intracellular concentrations of the parent drugs. In conjunction with the inhibition of anticancer drug glucuronidation, there was a concomitant formation of a glucuronide metabolite of propofol and an increase in its clearance from HT29 cells. We propose that these data are consistent with a mechanism of action where propofol prevents the generation of NU/ICRF 505 and SN-38 glucuronides by competitive inhibition of UGT, thus inhibiting clearance of the anticancer drugs from cells. Increased cellular retention of the anticancer drugs occurs, and as a consequence of more prolonged exposure, enhanced growth inhibitory activity (and reversal of intrinsic drug resistance) is observed (47, 48).

There are numerous previous reports demonstrating that propofol inhibits the activity of various cytochrome P-450s and UGTs in the liver and significantly in the intestine and decreases the clearance of a number of common drugs through competitive inhibition of drug metabolism (37, 49–51). In the current report, possibly the first of its kind, evidence is presented that propofol competitively inhibits the metabolism of two
anticycancer drugs in human colon cancer cells and selectively reverses intrinsic drug resistance.

A major finding of the present study was that human colon cancer biopsies could express levels of UGT protein in excess of those determined in HT29 cells but with marked interpatient variations and that the biopsies were highly proficient at catalyzing glucuronidation. Of the human colon cancer cell lines investigated, 50% had lost the capability to glucuronidate SN-38 and NU/ICRF 505 and did not express UGT protein. Among the latter were cell lines displaying a less well-differentiated phenotype (52). Conversely, induction of differentiation in HT29 cells has been shown to increase the expression of conjugating drug-metabolizing enzymes (53). The normal colon possesses a broader spectrum of members of the UGT1A locus than the liver or the upper gastrointestinal tract (16, 17) and a high percentage of colon cancer tumors retain well-differentiated morphology (52, 54). In a comparative study of esophageal tumors versus normal tissue, down-regulation of certain isoforms was noted (UGT1A7 and UGT1A10), whereas others (significantly UGT1A9) remained unaffected by malignant transformation (55). Thus, the ability of colon cancer cells to glucuronidate anticancer drugs would appear to be governed to a degree by differentiation status and the retention or up-regulation of the diverse drug-metabolizing phenotype of the normal colon, although environmental factors and the presence of polymorphisms also clearly contribute to the marked interpatient variations in glucuronidation activity observed in the present study and by others (35, 53, 56).

Hepatic UGTs play an important role in biliary excretion and detoxification of SN-38 through a reduction in the plasma area under the curve, whereas alterations in the capacity to perform glucuronidation by, for example, the expression of common polymorphic variants of the enzyme, can result in a dramatic increase in toxicity (57–60). Although, a relationship exists between plasma area under the curve of SN-38 and drug-induced bone marrow toxicity, a more complicated situation is envisaged in the case of late onset, dose-limiting diarrhea (61). It is now well recognized that intestinal toxicity is caused by the direct action of SN-38 on the luminal surface of epithelial cells (18, 62). Because only a small amount of native SN-38 is excreted in the bile, the majority of the compound accrues in the colon as its glucuronide (although a significant amount of native SN-38 is excreted in the bile, the majority of the compound is metabolized in the colon as its glucuronide (53)).

In summary, glucuronidation is now identified as a mechanism of intrinsic drug resistance. 

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