EPIRUBICIN GLUCURONIDATION IS CATALYZED BY HUMAN UDP-GLUCURONOSYLTRANSFERASE 2B7

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ABSTRACT:

Epirubicin is one of the most active agents for breast cancer. The formation of epirubicin glucuronide by liver UDP-glucuronosyltransferase (UGT) is its main inactivating pathway. This study aimed to investigate epirubicin glucuronidation in human liver microsomes, to identify the specific UGT isoform for this reaction, and to correlate epirubicin glucuronidation with other UGT substrates. Microsomes from human livers were used. UGTs specifically expressed in cellular systems, as well as two UGT2B7 variants, were screened for epirubicin glucuronidation. Epirubicin, morphine, and SN-38 glucuronides were measured by high-pressure liquid chromatography. The mean ± S.D. formation rate of epirubicin glucuronide in human liver microsomes (n = 47) was 138 ± 37 pmol/min/mg (coefficient of variation, 24%). This phenotype was normally distributed. We screened commercially available UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15 for epirubicin glucuronidation. Only UGT2B7 converted epirubicin to its glucuronide. No differences in epirubicin glucuronidation were found in HK293 cells expressing the two UGT2B7 variants at position 268. Catalytic efficiency (Vmax/Km) of epirubicin glucuronidation was 1.4 μl/min/mg, a value higher than that observed for morphine, a substrate of UGT2B7. Formation of epirubicin glucuronide was significantly related to that of morphine-3-glucuronide (r = 0.76, p < 0.001) and morphine-6-glucuronide (r = 0.73, p < 0.001). No correlation was found with SN-38, a substrate of UGT1A1 (r = 0.04). UGT2B7 is the major human UGT catalyzing epirubicin glucuronidation, and UGT2B7 is the candidate gene for this phenotype. The reported tyrosine to histidine polymorphism in UGT2B7 does not alter the formation rate of epirubicin glucuronide, and undiscovered genetic polymorphisms in UGT2B7 might change the metabolic fate of this important anticancer agent.

The topoisomerase II inhibitor epirubicin (4’-epi-doxorubicin) is a key component of chemotherapy for breast cancer patients, either in adjuvant or metastatic setting (Ormrod et al., 1999). Epirubicin produces similar efficacy with less adverse effects than its analog, doxorubicin, at equimolar doses (Ormrod et al., 1999). It is extensively metabolized by the liver, similar to other anthracyclines. Its 13-dihydro derivative, epirubicinol, has a very low degree of cytotoxicity, and aglycones of epirubicin and epirubicinol are considered minor inactive metabolites (Schott and Robert, 1989) (Fig. 1). Epirubicin has a different metabolic fate when compared with doxorubicin, as epirubicin and epirubicinol undergo conjugation with glucuronic acid by liver UDP-glucuronosyltransferase (UGT1) enzyme(s) (Weenen et al., 1984) (Fig. 1).

The main detoxifying pathway for epirubicin is the formation of epirubicin glucuronide (4’-O-β-D-glucuronyl-4’-epi-doxorubicin). Among epirubicin metabolites, epirubicin glucuronide is the major metabolite of the drug in plasma as well as in urine (Weenen et al., 1983). Mean area under the plasma concentration-time curve (AUC) values for epirubicin glucuronide were approximately 0.8 to 1.8 times those of the parent drug, while mean AUC values for epirubicinol and its glucuronide were approximately 0.2 to 0.6 times those of epirubicin (Weenen et al., 1983; Mross et al., 1988; Robert and Bui, 1992). Glucuronidation represents a protective mechanism to better eliminate lipophilic xenobiotics and endobiotics from the body, and epirubicin glucuronide is inactive, water soluble, and readily excreted in bile and urine (Camaggi et al., 1986).

The UGT isoform that glucuronidates epirubicin has not been identified. UGT enzymes are localized in the endoplasmic reticulum, and the human isoforms involved in drug metabolism are classified in UGT1 and UGT2 families based on sequence gene homology (Mackenzie et al., 1997). The glucuronidation pathway for epirubicin has been shown to be mainly limited to humans and has been investigated

1 Abbreviations used are: UGT, UDP-glucuronosyltransferase; AUC, area under the concentration-time curve; cDNA, complementary DNA; CN-I, Ogilier-Najjar syndrome type I; ECOD, 7-ethoxycoumarin O-deethylation; HPLC, high-pressure liquid chromatography; Tris, tris(hydroxymethyl)aminomethane; UDPGA, UDP-glucuronic acid; M0G, morphine-3-glucuronide; M6G, morphine-6-glucuronide.
Materials and Methods

Chemicals and Reagents. Epirubicin was kindly provided by Amersham Pharmacia Biotech (Milan, Italy). Bovine serum albumin, daunorubicin, β-glucuronidase, magnesium chloride, tris(hydroxymethyl)aminomethane (Tris), and UDP-glucuronic acid (UDPGA) were purchased from Sigma (St. Louis, MO). Acetonitrile, hydrochloric acid, methanol, ortho-phosphoric acid, and sodium dihydrogen phosphate were obtained from Fisher Scientific Co. (Fairlawn, NJ).

Microsomes Expressing Specific Human UGTs. Microsomes from human lymphoblasts and insect cells (BTI-TN-5B1-4) both transfected with a vector containing human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, and UGT2B15 complementary DNA (cDNA) and their negative control (microsomes from cells infected with wild-type vector) were obtained from GENTEST Corp. (Woburn, MA). Microsomes from insect cells (SF-9) infected with a baculovirus containing human cDNA for UGT2B7 and their negative control were purchased from PanVera Corp. (Madison, WI).

Preparation of Human Liver Microsomes and Measurement of 7-Ethoxycoumarin O-Deethylation Activity. Normal (nonpathologic) human livers (n = 47) were obtained through the Liver Tissue Procurement and Distribution System (National Institutes of Diabetes and Digestive and Kidney Diseases, Minneapolis, MN) after the approval of the Institutional Review Boards. Liver samples from Crigler-Najjar syndrome type I (CN-I) patients (n = 2) were obtained from Children’s Hospital and Queen Elizabeth Hospital (Birmingham, UK). Microsomes were prepared by differential centrifugation methods (Purba et al., 1987). Total protein content in microsomes was determined by the Bradford method using bovine serum albumin as the standard.

Microsomes from normal human livers (n = 47) were pooled for use in the optimization of glucuronidation reactions and kinetic analysis.

7-Ethoxycoumarin undergoes O-deethylation to umbelliferone by many different cytochrome P450s, and the metabolism of 7-ethoxycoumarin can serve as an index of the proper handling and storage of the liver tissue and preparation of microsomes. The measurement of 7-ethoxycoumarin O-deethylation (ECOD) activity in normal liver microsomes (n = 47) was performed as previously published, using a substrate concentration of 1 mM (Evans and Relling, 1992). ECOD activity in normal liver microsomes (n = 47) ranged from 1.4 to 18.5 nmol/h/mg, similar to that previously reported (Relling et al., 1992). This result led us to assume that the variability based on the storage and preparation of human liver microsomes was minimal. We avoided using detergents to activate microsomes because this could introduce a confounding factor in the assessment of catalytic activities in human liver microsomes. It has been shown that detergents not only increase membrane permeability, but they also affect enzyme activity itself, making UGTs intrinsically more active (Trapnell et al., 1998).

Epirubicin Glucuronidation Assay. A typical incubation consisted of final concentrations of epirubicin (200 μM), magnesium chloride (10 mM), total microsomal protein (3 mg/ml), and Tris-HCl buffer (0.1 M, pH 7.4) in a total volume of 100 μl. The incubations used an epirubicin concentration of 200 μM to ensure relevance to the clinical use of this agent. Peak plasma concentrations up to 6 μM have been observed in cancer patients, and epirubicin is extensively distributed (volume of distribution = 32–46 l/m²), reaching higher concentrations in tissues than in plasma (Ormrod et al., 1999). Depletion of the substrate (if any) seems to be minimal and to not influence the enzyme kinetics, since, during the optimization process, we observed that the production of epirubicin glucuronide was linear up to 4 h of incubation with 10 μM epirubicin and 1 mg/ml of microsomes (data not shown).

All mixtures were preincubated for 5 min at 37°C to achieve thermal equilibrium, and the reaction was initiated by adding UDPGA (5 mM). After 4 h of incubation in a shaking water bath at 37°C, the reaction was stopped with 0.4 ml of cold methanol. After the addition of 10 μl of the internal
standard (daunorubicin, 1 nmol), samples were shaken for 20 min and centrifuged at 14,000 rpm for 30 min. The supernatant was dried under nitrogen at 37°C, and samples were resuspended with 200 μl of mobile phase. After centrifugation at 14,000 rpm for 15 min, the supernatant was injected into the high-pressure liquid chromatography (HPLC) system. Control reactions without epirubicin, microsomes, and UDPGA were simultaneously performed. Hydrolysis with β-glucuronidase was used to identify the epirubicin glucuronide peak. For this purpose, dried samples were reconstituted with 0.2 ml of sodium phosphate buffer (0.1 M, pH 6.8) containing 1000 U of β-glucuronidase (type VII, from Escherichia coli) and incubated overnight at 37°C. Reference samples containing no enzyme were treated identically. The reaction was stopped with 0.4 ml of cold methanol, and the two sets of samples were then analyzed as described below.

Because pure epirubicin glucuronide was unavailable, this metabolite was quantitated by comparing measured peak heights to those of a standard curve generated for unchanged epirubicin. Fluorescence of epirubicin glucuronide was assumed to be equal to epirubicin based on their fluorescence spectra, similar to findings from other studies (Barker et al., 1996). The concentrations of epirubicin glucuronide were determined using a HPLC system (Hitachi Instruments, San Jose, CA) with fluorescence detection at 480 (λex) and 560 (λem) nm. Epirubicin, its glucuronide, and daunorubicin were separated using a reversed phase Supelcosil LC-CN column (5 μm, 4.6 × 250 mm, Supelco Inc., Bellefonte, PA) preceded by a μBondapak LC-CN guardpak (Waters Corp., Milford, MA). The mobile phase consisted of 30% acetonitrile and 70% 50 mM sodium dihydrogen phosphate (pH adjusted to 4 with 8.5% ortho-phosphoric acid). At a flow of 0.8 ml/min, the retention times of epirubicin glucuronide, epirubicin, and daunorubicin were 5.7, 7.4, and 10.1 min, respectively. Standard curves for epirubicin were linear within the range of 5 to 800 μM. Inter-assay reproducibility was analyzed by incubating three pooled liver microsomal samples each day for 3 days, and the coefficient of variation was less than 5%. Intra-assay reproducibility was obtained by measuring epirubicin glucuronide formation in 10 separate incubations of the same batch of pooled liver microsomes, and the coefficient of variation was less than 5%.

**Morphine Glucuronidation Assay.** A typical incubation consisted of final concentrations of morphine (1.4 mM), magnesium chloride (5 mM), total microsomal protein (2 mg/ml), and Tris-HCl buffer (0.1 M, pH 7.4) in a total volume of 100 μl. After 5 min of preincubation at 37°C, the reaction was initiated by adding UDPGA (5 mM). After 20 min of incubation in a shaking water bath at 37°C, the reaction was stopped with 0.4 ml of cold acetonitrile. After the addition of 10 μl of the internal standard (10,11-dihydrocarbamazepine, 42 nmol), samples were shaken for 20 min and centrifuged at 14,000 rpm for 30 min. The supernatant was dried under nitrogen at 37°C, and samples were resuspended with 200 μl of mobile phase. After centrifugation at 14,000 rpm for 15 min, the supernatant was injected into the HPLC system. Control reactions without morphine, microsomes, and UDPGA were similarly performed. The concentrations of morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) were determined by HPLC with fluorescence detection at 210 (λex) and 340 (λem) nm. Morphine, M3G, M6G, and 10,11-dihydrocarbamazepine were separated using a reversed phase Bondapak C18 column (10 μm, 3.9 × 300 mm, Waters Corp.) preceded by a Novapak C8 guardpak (Waters Corp.). The mobile phase consisted of 25% acetonitrile and 75% 10 mM sodium dihydrogen phosphate and 1 mM sodium dodecyl sulfate (pH adjusted to 2.1 with 85% ortho-phosphoric acid). At a flow of 1 ml/min, the retention times of M3G, M6G, morphine, and 10,11-dihydrocarbamazepine were 8.9, 11.5, 17.1, and 27.7 min, respectively. Standard curves for M3G and M6G were linear within the range of 1 to 125 μM and 1 to 50 μM, respectively. Inter-assay reproducibility was analyzed by incubating three pooled liver microsomal samples each day for 3 days, and the coefficient of variation was 6.3 and 8.7% for M3G and M6G, respectively. Intra-assay reproducibility was obtained by measuring epirubicin glucuronidation formation in 10 separate incubations of the same batch of pooled liver microsomes, and the coefficient of variation was 5.7 and 9.4% for M3G and M6G, respectively.

**SN-38 Glucuronidation Assay.** To investigate whether the correlation epirubicin/morphine were specific for UGT2B7 and not related to the UGT family in general, the glucuronidation of SN-38, a substrate of UGT1A1, has been correlated to that of epirubicin. We measured glucuronidation rates of SN-38 in normal human liver microsomes (n = 47) as previously described (Iyer et al., 1998a).

<table>
<thead>
<tr>
<th>Source</th>
<th>Epirubicin Glucuronide</th>
</tr>
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<tbody>
<tr>
<td>Normal livers</td>
<td>138 ± 37</td>
</tr>
<tr>
<td>CN-I n. 1</td>
<td>144 ± 6</td>
</tr>
<tr>
<td>CN-I n. 2</td>
<td>104 ± 6</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B15</td>
<td>N.D.</td>
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N.D., not detectable.

**Epirubicin Glucuronidation in HK293 Cell Membranes Expressing UGT2B7(H) and UGT2B7(Y) Variants.** Two UGT2B7 variants have been identified, differing for a single amino acid change, i.e., tyrosine for histidine in UGT2B7(Y) and UGT2B7(H), respectively (Jin et al., 1993b). To test for possible differences in epirubicin glucuronidation rates between the two UGT2B7 variants, HK293 cells transfected with human cDNA and specifically expressing UGT2B7(Y) and UGT2B7(H) were used. Stable expression of human UGT2B7(Y) and UGT2B7(H) was obtained as previously described (Coffman et al., 1997). Membranes from HK293 cells were prepared according to the method described by King et al. (1997). Incubation conditions were those adopted for human liver microsomes.

**Data Analysis and Statistics.** Results are presented as mean ± S.D. of a single experiment performed in triplicate. To describe the formation rate of epirubicin glucuronide, pooled liver microsomes and UGT2B7 microsomes were separately incubated in the presence of a substrate range of 50 to 1000 μM, while the concentration of UDPGA was held constant (5 mM). Kinetics of conjugation reactions for morphine has been evaluated as well, and substrate concentration was varied from 0.2 to 10 mM. Two separate experiments in triplicate were performed. Data were analyzed by simple hyperbolic function (with r2 indicating the goodness of fit), and apparent Km and Vmax values of the reactions were estimated (GraphPad software, GraphPad Software Inc., San Diego, CA). Catalytic efficiencies (Vmax/Km) were also calculated. The Pearson correlation coefficient was adopted to test the level of correlation between epirubicin and other UGT substrates like morphine and SN-38, and the cut-off for statistical significance was set at 0.05. Frequency distribution of epirubicin glucuronidation in 47 microsomal preparations from normal human livers was described.

**Results**

**Optimization of Epirubicin and Morphine Glucuronidation Reaction.** Optimal assay conditions were established using pooled liver microsomes. Variables such as incubation time, microsomal protein content, and UDPGA concentrations were examined. The enzymatic reaction was shown to be linear up to 30 min and 4 h of incubation for morphine and epirubicin, respectively. Maximal rates of morphine and epirubicin glucuronidation were obtained with a microsomal protein concentration of 2 and 3 mg/ml, respectively. Increases in UDPGA concentration from 5 to 15 mM did not significantly change the production of glucuronidated metabolites of both drugs, and an UDPGA concentration of 5 mM was adopted.

**Epirubicin Glucuronidation in Normal and CN-I Liver Microsomes.** The formation rate of epirubicin glucuronide in normal liver microsomes was 138 ± 37 (mean ± S.D.) pmol/min/mg (n = 47) (Table 1). A coefficient of variation of 24% and a 4-fold difference were observed. To identify the possible contribution of UGT1A1 to epirubicin glucuronidation, the formation of epirubicin glucuronide was measured in CN-I liver microsomes. Glucuronidating activity of UGT1A1 is genetically absent in patients affected by CN-I, a severe unconjugated hyperbilirubinemia (Seppen et al., 1994). In liver mi-
In pooled human liver microsomes and in microsomes of epirubicin glucuronide as a function of substrate concentration was measured in pooled human liver microsomes and in microsomes expressing UGT2B7 (Fig. 2, A and B). Both reactions followed Michaelis-Menten kinetics of glucuronidation of epirubicin by normal liver microsomes (A) and UGT2B7 microsomes (B).

Since morphine is glucuronidated by UGT2B7 (Coffman et al., 1997), correlation between epirubicin and morphine glucuronidation rates was assessed in 47 normal human liver microsomes. Formation of epirubicin glucuronide was significantly related to that of M3G (r = 0.76, p < 0.001) and M6G (r = 0.73, p < 0.001) (Fig. 4, A and B, respectively). Correlation of glucuronidation rates between epirubicin and SN-38, the active metabolite of irinotecan and UGT1A1 substrate (Iyer et al., 1998a) was investigated. No correlation was observed with SN-38 glucuronidation (r = 0.04) (Fig. 4C).

**Discussion**

This paper provides the first experimental finding of the involvement of UGT2B7 in epirubicin glucuronidation. We have provided evidence for this by demonstrating epirubicin glucuronidation in cell systems specifically expressing the UGT2B7 isof orm, and by a correlation study in human liver microsomes using morphine as the probe drug for UGT2B7.

We have screened for epirubicin glucuronidation the five functional UGT1A isoforms expressed in hepatic tissue and, under the present experimental conditions, none of them was able to catalyze epirubicin glucuronidation. Epirubicin glucuronidation activity in all commercially available microsomes expressing specific UGT isoforms revealed that epirubicin was glucuronidated only by UGT2B7. No epirubicin glucuronidating activity was observed in microsomes from cells expressing UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, and UGT2B15 (Table 1). Most of the microsomal preparations of expressed UGTs in the present study have been used for other purposes, showing functional activity toward other substrates. For the UGTs that have not been tested, we assumed they are active because of company guarantee.

The formation rate of epirubicin glucuronide by cDNA-expressed UGT2B7 was 63 ± 4 pmol/min/mg (Table 1). There was no glucuronidation of epirubicin in control microsomes from cells infected with wild-type vector. The epirubicin glucuronide peak produced by cDNA-expressed UGT2B7 was further confirmed by treatment with β-glucuronidase enzyme, which resulted in the loss of the glucuronide (data not shown). Differences in epirubicin glucuronidation between UGT2B7(H) and UGT2B7(Y) variants were not observed, with mean ± standard error values of 0.762 ± 0.037 and 0.743 ± 0.047 epirubicin glucuronide/internal standard, respectively.

**Kinetic Parameters and Frequency Distribution of Epirubicin Glucuronidation in Human Liver Microsomes.** The formation rate of epirubicin glucuronide as a function of substrate concentration was measured in pooled human liver microsomes and in microsomes expressing UGT2B7 (Fig. 2, A and B). Both reactions followed Michaelis-Menten kinetics (r² = 0.99). In human liver microsomes, apparent Kₘ and Vₘₐₓ values were 568 ± 130 µM and 798 ± 87 pmol/min/mg (mean ± standard error), respectively. In microsomes expressing UGT2B7, apparent Kₘ and Vₘₐₓ values were 149 ± 22 µM and 99 ± 4 pmol/min/mg (mean ± standard error), respectively. Catalytic efficiencies (Vₘₐₓ/Kₘ ratios) were 1.4 and 0.66 µl/min/mg for liver microsomes and microsomes expressing UGT2B7, respectively. This apparent difference can be explained by differences in lipid composition of microsomal membranes and amount of functional enzyme (Remmel and Burchell, 1993), as well as by the involvement of another UGT not yet tested. However, the correlation study with morphine, the probe drug for UGT2B7, seems to exclude the significant contribution of other UGTs to epirubicin glucuronidation.

Frequency distribution analysis of epirubicin glucuronidation rates in 47 normal human liver microsomes showed that this phenotype is apparently normally distributed (Fig. 3). The median value of epirubicin glucuronidation rates was 136 pmol/min/mg, a value very close to the mean value (138 pmol/min/mg).

**Kinetic Parameters of Morphine Glucuronidation in Human Liver Microsomes.** The M3G and M6G glucuronidation rates were 1.25 ± 0.46 and 0.19 ± 0.06 nmol/min/mg (mean ± S.D.), with coefficients of variations of 37 and 32%, respectively. The M3G and M6G ratios were 6.55 ± 0.89 (coefficient of variation = 13%), and the correlation coefficient between M3G and M6G was 0.92 (p < 0.001). Both M3G and M6G formation followed Michaelis-Menten kinetics (r² = 0.99 and 0.97 for M3G and M6G, respectively; data not shown). With regard to M3G, apparent Kₘ and Vₘₐₓ values were 1988 ± 225 µM and 1549 ± 66 pmol/min/mg (mean ± standard error), respectively. With regard to M6G, apparent Kₘ and Vₘₐₓ values were 1869 ± 356 µM and 215 ± 15 pmol/min/mg (mean ± standard error), respectively. Catalytic efficiencies were 0.78 and 0.11 µl/min/mg for M3G and M6G, respectively (Table 2).
This suggests that the UGT1A family is unlikely to be involved in this conjugation reaction in the liver. Moreover, we observed similar formation rates of epirubicin glucuronide in normal and CN-I liver microsomes. Our results suggest that epirubicin glucuronidation would be unaffected in subjects with Gilbert’s syndrome, a common hyperbilirubinemia caused by genetically decreased UGT1A1 activity (Monaghan et al., 1996). This is in agreement with the absence of major changes in the pharmacokinetics of epirubicin in one patient affected by Gilbert’s syndrome (Riggi et al., 1999).

The human UGT2B family comprises six human UGT2B isoforms, with two of them lacking any substrate specificity (i.e., UGT2B10 and UGT2B11) (Radominska-Pandya et al., 1999). No formation of epirubicin glucuronide was observed with UGT2B15, while epirubicin was glucuronidated in microsomes specifically expressing UGT2B7. The kinetic analysis of epirubicin and morphine glucuronidation in human liver microsomes showed higher catalytic efficiency for epirubicin in comparison with morphine. Since UGT2B7 is highly expressed in the liver (Jin et al., 1993a), this observation suggests that UGT2B7 might contribute significantly to the glucuronidation of epirubicin. Moreover, in agreement with our data, epirubicin significantly inhibited the in vitro glucuronidation of the anticancer drug 5,6-dimethylxanthenone-4-acetic acid, a substrate of UGT2B7 (Miners et al., 1997).

Many drugs and endogenous substrates are glucuronidated by UGT2B7. Among them, UGT2B7 catalyzes morphine glucuronidation at 3-OH and 6-OH positions, leading to the formation of M3G and M6G, respectively (Coffman et al., 1997). Our correlation study shows that epirubicin glucuronidation is related to that of morphine. Although M6G formation has been considered quite specific for UGT2B7 since glucuronidation at the 3-OH position occurs by UGT1A1 and UGT1A3 (King et al., 1996; Green et al., 1998), we actually found the formation of M3G and M6G to be highly correlated. In addition to UGT2B7, different UGTs with a minor role in morphine glucuronidation at the 6-OH position could have slightly interfered with the correlation between epirubicin glucuronide and M6G. To our knowledge, a systematic screening of all the liver UGTs potentially involved in morphine glucuronidation has never been conducted.

Concerning UGT2B7 polymorphism, two UGT2B7 variants with a substitution of tyrosine for histidine at codon 268 have been classified as UGT2B7(Y) and UGT2B7(H), respectively (Jin et al., 1993b; Coffman et al., 1998). This single amino acid change arises from a C to T transition at nucleotide 802 of UGT2B7 gene (Jin et al., 1993b). Our study indicated that this amino acid difference does not alter the glucuronidation rate of epirubicin. With the exception of buprenorphine, this polymorphism does not seem to have any functional significance for bile acids, estrogens, androgens, opioids, and zidovudine (Hashiguchi et al., 1995; Coffman et al., 1998; Gall et al., 1999).

In cancer chemotherapy, reduced drug glucuronidation in patients has been shown to be an important determinant for prediction of toxicity (Miners et al., 1997).
toxicity (Gupta et al., 1994; Innocenti et al., 2000; Iyer et al., 2000). The number of anticancer agents in development undergoing glucuronidation by polymorphic UGTs is rapidly increasing (Gupta et al., 1994; Iyer et al., 1998a,b). As genetic differences in metabolic drug inactivation can affect both the pharmacokinetic and clinical outcomes for cancer patients, strategies of phenotyping and/or genotyping could be adopted to identify patients genetically predisposed to severe toxicities.

Similar to other anticancer drugs, epirubicin pharmacokinetics are quite variable, which can lead to serious clinical consequences ( Dobbs and Twelves, 1998). The degree of myelosuppression is highly related to the AUC of epirubicin ( Jakobsen et al., 1991; Dobbs and Twelves, 1998), and an almost 10-fold interpatient variability in AUC values has been reported ( Eksborg, 1989). Functional polymorphisms in UGT2B7 gene resulting in significant changes in enzyme catalytic activity could either reduce or increase the amount of epirubicin circulating in the bloodstream. For this reason, part of the variable exposure of patients to epirubicin could be explained by genetically determined differences in hepatic UGT2B7 activity.

In a recent study of sequential epirubicin and paclitaxel, significant reduction in epirubicin glucuronide AUC in the sequence paclitaxel—epirubicin could have led to less efficient and lower elimination of epirubicin with respect to the sequence epirubicin—paclitaxel ( Venturini et al., 2000). This provides further evidence for the importance of this inactivating pathway for epirubicin disposition in cancer patients, since the production of epirubicin glucuronide could divert epirubicin from its hydroxylation to epirubicinol. Intracellular formation of hydroxylated anthracyclines results in toxic damage to cardiac cells ( Minotti et al., 1995), and since epirubicinol is glucuronidated as well, this pathway could be regarded as a protective mechanism.

The pharmacodynamic significance of epirubicin glucuronidation in the clinical setting has not been studied in detail. In one study, a lower systemic production of epirubicin glucuronide coupled to a higher availability of the parent compound was associated with a better hematologic tolerance and response ( Robert et al., 1990). Interestingly, metabolic ratios between epirubicin glucuronide/epirubicin AUCs in cancer patients are bimodally distributed ( Robert et al., 1990). The expression of mutated UGT2B7 alleles encoding enzyme variant isoforms with reduced activity might explain the existence of poor and extensive glucuronidators of epirubicin.

Our data indicate that UGT2B7 is the major UGT catalyzing epirubicin glucuronidation and that UGT2B7 can be regarded as the candidate gene for this phenotype. Phenotyping of epirubicin glucuronidation could be investigated by using morphine as a probe drug. Studies are in progress to characterize the genetic basis of variability in epirubicin glucuronidation. Future studies will evaluate the relationship between polymorphisms in UGT2B7 and epirubicin pharmacokinetics and pharmacodynamics.

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### References


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**Fig. 4.** Correlation analysis between formation rates of epirubicin glucuronide versus those of M3G (A), M6G (B), and SN-38 glucuronide (C) in 47 normal human liver microsomes.

Epirubicin glucuronidation is significantly related to that of M3G (r = 0.76, p < 0.001) and M6G (r = 0.73, p < 0.001). No evidence of correlation is observed between epirubicin and SN-38, a substrate of UGT1A1 (r = 0.04).


