GLUCURONIDATION: AN IMPORTANT MECHANISM FOR DETOXIFICATION OF BENZO[a]PYRENE METABOLITES IN AERODIGESTIVE TRACT TISSUES

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ABSTRACT:

UDP-glucuronosyltransferases (UGTs) have been implicated as important detoxifying enzymes for several major tobacco carcinogens. Because the aerodigestive tract is a primary target for exposure to tobacco smoke carcinogens, the major goal of the present study was to determine whether aerodigestive tract tissues exhibit glucuronidating activity against metabolites of benzo[a]pyrene (BaP) and to explore the pattern of expression of UGT genes in a series of aerodigestive tract tissue specimens. Glucuronidation of the phenolic BaP metabolites 3-, 7-, and 9-hydroxy-BaP was observed in all upper aerodigestive tract tissue specimens. Of the aerodigestive tract-expressing UGTs, only UGTs 1A7 and 1A10 exhibited glucuronidating activity against 7-hydroxy-BaP, with UGT1A10 exhibiting the highest affinity as determined by kinetic analysis (Km = 49 μM). No UGT expression or glucuronidating activity was observed for any of the lung specimens analyzed in this study. These results suggest that several family 1 UGTs may potentially play an important role in BaP detoxification in the aerodigestive tract.

The UGT2 superfamily of enzymes catalyze the glucuronidation of a variety of compounds, including endogenous compounds like bilirubin and steroid hormones, as well as xenobiotics including drugs and environmental carcinogens (Tephly and Burchell, 1990; Guerard and Paris, 1998; Ren et al., 2000). Based upon differences in sequence homology and substrate specificity, two main families of UGTs (UGT1A and UGT2B) have been identified in several species, each containing several highly homologous UGT genes. The entire UGT1 family is derived from a single locus in chromosome 2, coding for nine functional proteins that differ only in their amino terminus due to alternate splicing of the independent exon 1 regions to a shared carboxy terminus encoded by exons 2 to 5 (Owens and Ritter, 1995). In contrast to the UGT1A family, the UGT2B family is composed of several independent genes, all located on chromosome 4 (Jin et al., 1993a,b; Beaulieu et al., 1997, 1998; Belanger et al., 1998; Carrier et al., 2000).

In previous studies, several UGTs, including UGT2B7, UGT1A9, UGT1A7, UGT1A8, and UGT1A10, were implicated in the conjugation and detoxification of metabolites of several tobacco carcinogens, including tobacco-specific nitrosamines like NNK (Ren et al., 2000), and polycyclic aromatic hydrocarbons, such as BaP (Jin et al., 1993a,b; Grove et al., 1997; Mojjarrabi and Mackenzie, 1998; Strassburg et al., 1999; Guillemette et al., 2000). In addition, several studies have demonstrated that UGTs exhibit a protective effect against these carcinogens. The addition of UDPGA to the Ames test is associated with a reduction in BaP mutagenicity (Nemoto et al., 1978; Owens et al., 1999; Beaulieu et al., 1997, 1998; Belanger et al., 1998; Carrier et al., 2000). In studies of UGT-deficient homozgous (jf) and heterozygous (jf+) RHA rats versus UGT-normal (+/+) RHA controls, reduced glucuronidation of BaP metabolites in vivo was correlated with increased covalent binding to hepatic DNA and microsomal protein (Hu and Wells, 1992). In addition, a similar correlation was observed after in vitro incubations of BaP with rat liver microsomes, lymphocytes, or skin fibroblasts from UGT-deficient RHA rats (Hu and Wells, 1992; 1994; Vienneau et al., 1995). Therefore, several UGT enzymes could potentially play an important role in the detoxification of tobacco carcinogens.
Although studies examining UGT expression patterns in human tissues have been performed extensively for metabolizing organs and tissues of the digestive tract (Strassburg et al., 1998a,b, 1999, 2000), few studies have been performed for tobacco-related target tissues. For tissues of the aerodigestive tract and respiratory system, UGT1A7 was shown to be well expressed in orolaryngeal specimens (Zheng et al., 2001), and UGT1A6 was shown to be expressed in pharyngeal tissue (Ulrich et al., 1997). Family 2B UGTs were reported to be expressed in lung (Levesque et al., 1997, 1999; Hum et al., 1999), whereas several UGTs (UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B10, and UGT2B15) were reported to be expressed in esophagus (Strassburg et al., 1999). No data have as yet been reported demonstrating glucuronidating activity in such target organ sites. To better assess the role of glucuronidation as a detoxification mechanism in tissues of the aerodigestive tract, the goal of the present study was to determine whether aerodigestive tract tissues exhibit glucuronidating activity to BaP metabolites and to examine the pattern of expression of UGT genes in aerodigestive tract tissues.

Materials and Methods

Tissues. For expression analysis, total RNA purified from normal human liver (19 samples), lung (32 samples), esophagus (10 samples), larynx (three samples), tongue (five samples), tonsil (three samples), and floor of mouth (three samples) specimens was obtained from the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center. All RNA samples were purified from tissue specimens obtained from individual subjects undergoing cancer surgery. Accurate information on recent exposures (i.e., smoking and alcohol consumption) was not available for this study.

For glucuronidation activity assays, larynx (n = 4), floor of mouth (n = 2), tongue (n = 2), esophagus (n = 3), tonsil (two specimens from the same patient), and lung (n = 3) specimens were obtained from individual patients via the H. Lee Moffitt Cancer Center Tissue Procurement Facility. Because of the low quantities of normal orolaryngeal tissues obtained during cancer surgery, equal amounts of each specimen from each orolaryngeal site were pooled for the preparation of microsomes. Because larger specimens were obtained for lung, analysis of glucuronidation activity was performed separately in three independent lung specimens. Microsomes were prepared for all specimens by differential centrifugation, as previously described (Coughtrie et al., 1994). The HPLC flow rate was 1 ml/min, whereas the scintillation fluid flow rate was 4 ml/min. The column was routinely washed with 100% A for 15 min and equilibrated after every HPLC run with 20% A for at least 20 min. Glucuronidated conjugates of BaP metabolites were verified by sensitivity of individual reactions to Escherichia coli β-glucuronidase treatment (1000 units, 37°C, 16 h) using HPLC, as described above. Glucuronidation activities were calculated based on radioflow detection and quantification of disintegrations per minute within glucuronidated BaP metabolite-specific HPLC peaks, as determined using the IN/US radioactivity detection program.

Analysis of Glucuronidating Activity of UGT-Overexpressing Cell Lines, Microsomes, or Baculosomes. HK293 (human embryonic kidney fibroblast) cells and HK293 cell lines overexpressing UGT1A8 were kindly provided by Dr. Thomas Tephy (University of Iowa, Iowa City, IA; Cheng et al., 1998), whereas V79 (Chinese hamster fibroblast) cells and V79 cells overexpressing UGT1A6 were kindly provided by Dr. Brian Burchell (University of Dundee, Scotland, UK; Ebner and Burchell, 1993). The stable transfectant of the UGT2B4-overexpressing cell line has been described previously (Ren et al., 2000). UGT2B17-overexpressing cell microsomes were kindly supplied by Chantal Guillemette (University of Laval, Quebec City, Canada). All V79 and HK293 cell lines were grown to 80% confluency in Dulbecco’s modified Eagle’s medium supplemented with 4.5 mM glucose, 10 mM HEPES, 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin and maintained in 700 µg/ml geneticin for selection of UGT over-expression, in a humidified incubator under an atmosphere of 5% CO₂.

Cells were suspended in Tris-buffered saline (25 mM Tris base, 138 mM NaCl, and 2.7 mM KCl, pH 7.4) and subjected to 3 rounds of freeze-thaw before gentle homogenization. Cell homogenates (5–30 mg of homogenate protein/ml) were stored at −70°C in 100-µl aliquots. Total cell homogenate protein concentrations were determined using the bichinchoninic acid assay, as described above.

The rate of 7-OH-BaP glucuronidation was determined in individual UGT enzymes as described above for tissue microsomes in assays without detergent using 0.1 to 5 mg of UGT-overexpressing cell homogenate, 20 µg of UGT-overexpressing microsomal protein, or 0.1 mg of UGT-overexpressing baculoso- lase protein. Initial activity screenings were performed at 37°C for 16 h. Kinetic analysis for all UGTs exhibiting significant glucuronidating activity against 7-OH-BaP was performed as described above using an incubation time of 2 h (where the rate of BaP→7-O-glucuronide formation was still linear for each UGT enzyme tested; results not shown). The Kₘ and Vₘₚₐₜ for the glucuronidation of 7-OH-BaP by individual UGT enzymes were calculated after linear regression analysis of Lineweaver-Burk plots. For UGT-overexpressing cell homogenate or microsomal experiments, the parent HK293 or V79 cell lines served as negative controls for all in vitro glucuronidation reactions.

To confirm that all UGT-overexpressing cell homogenates, microsomes, and baculosomes tested in this study were active, glucuronidation assays were performed with known test substrates. Thin layer chromatography analysis was performed as described previously (Ren et al., 1999, 2000) for glucuronidation assays using 2 mM either 1-naphthol (UGT1A6), clofibric acid (UGT2B4), or androsterone (UGT2B17) as a test substrate. As with 7-OH-BaP glucuronidation analysis, glucuronide formation for these test substrates was confirmed by treatment with E. coli β-glucuronidase, as described above.

Duplex RT-PCR. RT was performed in 20-µl volumes using 3 µg of total RNA, 200 units Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA), and 0.5 µg of oligo (dT)₁₅ primer, as outlined in the manufacturer’s protocol. Equal amounts of total RNA from each specimen was used for the analysis of pooled RNA samples. For PCR (a 50-µl final volume), each reaction was performed using 5 µl of RT reaction, 0.2 mM dNTPs, 5 U Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN), 2.5 mM MgCl₂, 1× PCR buffer (Boehringer Mannheim), and 20 pmol of sense and antisense
with each PCR performed in duplicate experiments. For semiquantitative RT-PCR analysis, aliquots were removed from sense and antisense primers (20 pmol each) were added to PCRs after the ninth cycle. Purification System; Promega Corp., Madison, WI) to confirm the UGT purified RT-PCR products were directly sequenced (Molecular Core Facility, RT-PCR products were performed using a computerized photoimager system for expression analysis of all family 1A UGT genes. For the initial screening was observed in human lung microsomes using as much as 1 mg of microsomal protein for these tissues due to the relatively small amount of specific for exon 1 sequences in each of the respective UGT genes. The same exon 3-derived antisense primer was used for RT-PCR amplifications of UGT2B4 and UGT2B7. The same exon 2-derived antisense primer was used for RT-PCR amplifications of UGT2B15 and UGT2B17.

Results

Glucuronidation Activity of Aerodigestive Tract Microsomes toward BaP Metabolites. As a marker to evaluate whether aerodigestive tract tissues exhibit glucuronidating activity against metabolites of BaP, studies were initially performed using 7-OH-BaP as substrate. As shown in Fig. 1, significant levels of BaP-7-O-Gluc was detected in glucuronidation assays using pooled samples of human esophageal microsomes. BaP-7-O-Gluc formation in esophageal microsomes was detected by both UV detection (254 nm; Fig. 1A) and UDPGA-derived [14C]glucuronic acid incorporation (Fig. 1C), and the predicted BaP-7-O-Gluc peak on HPLC was sensitive to treatment with β-glucuronidase (Fig. 1, B and D). The rate of glucuronidation of 7-OH-BaP for esophageal microsomes (16.4 nmol · mg of protein \(^{-1}\) · 120 min \(^{-1}\); Table 2) was similar to that observed for liver microsomes (21.2 nmol · mg of protein \(^{-1}\) · 120 min \(^{-1}\); results not shown). High levels of BaP-7-O-Gluc were formed in assays of pooled microsomes (0.1 mg) from all other aerodigestive tract tissues tested, including larynx, tonsil, tongue, and floor of mouth (Table 2). No difference in activities were observed for any tissue microsomes in assays with or without DL-2-lyso phosphatidyl choline palmityl (C16:0). No activity was observed in human lung microsomes using as much as 1 mg of microsomal protein in assays with or without DL-2-lyso phosphatidyl choline palmityl (C16:0).

Similar to that observed for 7-OH-BaP, high levels of glucuronidating activity were observed against both 3-OH-BaP and 9-OH-BaP with all aerodigestive tract tissues tested (Table 2). All aerodigestive tract tissue microsomes except larynx exhibited activity in the order of 7-OH-BaP > 3-OH-BaP > 9-OH-BaP.

Significant levels of glucuronidating activity were also detected against trans-BaP-7,8-dihydrodiol (±) for pooled microsomes (1 mg of protein) from both laryngeal and esophageal tissues (Table 2). No glucuronidating activity was detected against trans-BaP-7,8-dihydrodiol (±) in tonsil microsomes using up to 1 mg of microsomal protein. Glucuronidation of trans-BaP-7,8-dihydrodiol (±) was also not detected in pooled microsomes from both tongue and floor of mouth, but assays were performed using only 0.1 mg of microsomal protein for these tissues due to the relatively small amount of specimen obtained for each of these sites. Similar to that observed for 7-OH-BaP, no activity was detected against 3-OH-BaP, 9-OH-BaP, or trans-BaP-7,8-dihydrodiol (±) in assays of human lung microsomes using up to 1 mg of microsomal protein (Table 2).

Expression of UGT mRNA. To evaluate UGT gene expression in aerodigestive tract tissues, duplex RT-PCR analysis was performed for pooled total RNA samples prepared from multiple normal human tongue, tonsil, floor of mouth, larynx, and esophagus, as well as from normal human lung and liver specimens. Of the family 1 UGTs (Fig. 2A), UGT1A6, UGT1A7, and UGT1A10 were all expressed in aerodigestive tissues but not in lung. Similar to that observed by Strassburg et al. (1999), UGT1A7, UGT1A8, and UGT1A10 were not

<table>
<thead>
<tr>
<th>Primer (^a)</th>
<th>Sequence</th>
<th>GenBank Access Number</th>
<th>Location (^b)</th>
<th>Expected Size</th>
</tr>
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<tr>
<td>UGT1A1 sense</td>
<td>5’-aacaaagggctaatgctatgccccc</td>
<td>NM_000463</td>
<td>412–432</td>
<td>646</td>
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<tr>
<td>UGT1A3 sense</td>
<td>5’-tggtgacacatatgctgtagttca</td>
<td>NM_019093</td>
<td>347–371</td>
<td>698</td>
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<tr>
<td>UGT1A4 sense</td>
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<td>AF297903</td>
<td>135686–135706</td>
<td>787</td>
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<tr>
<td>UGT1A5 sense</td>
<td>5’-ggtggtgctctcccccgtt</td>
<td>AF297903</td>
<td>129970–129988</td>
<td>701</td>
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<tr>
<td>UGT1A6 sense</td>
<td>5’-tcctcggtgcttgatggtggcc</td>
<td>NM_001072</td>
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<tr>
<td>UGT1A7 sense</td>
<td>5’-agtgcctgctgcggccacatt</td>
<td>U98507</td>
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<tr>
<td>UGT1A8 sense</td>
<td>5’-gtcctctgcggagggataagg</td>
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<td>UGT1A9 sense</td>
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<td>AF056188</td>
<td>660–683</td>
<td>391</td>
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<td>UGT1A10 sense</td>
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<td>U98508</td>
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<td>477</td>
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<td>UGT1A antisense (^c)</td>
<td>5’-ggctgcccaagtccgtgcttg</td>
<td>AF297903</td>
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<td>UGT2B4 sense</td>
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<td>NM_021139/NM_001074</td>
<td>249–273</td>
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<td>UGT2B7 sense</td>
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<td>NM_001074</td>
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<td>592</td>
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<tr>
<td>UGT2B4/7 antisense (^c)</td>
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<td>NM_021139/NM_001074</td>
<td>954–935 (UGT2B4)</td>
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<td>UGT2B15 sense</td>
<td>5’-gggagattattagctgacagc</td>
<td>NM_001076</td>
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<td>UGT2B17 sense</td>
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<td>NM_001077</td>
<td>398–422</td>
<td>519</td>
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<tr>
<td>UGT2B15/17 antisense (^c)</td>
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<td>887–869 (UGT2B15)</td>
<td>915–899 (UGT2B17)</td>
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</table>
expressed in human liver. UGT1A8 was detected specifically in larynx but not in other aerodigestive tract tissues or in lung. Although significant levels of expression was observed in liver, no expression of UGT1A1, UGT1A3, UGT1A4, or UGT1A9 was detected in lung or in any of the aerodigestive tissues examined using pooled RNA samples (Fig. 2A) or RNA from individual specimens (results not shown).

Similar to that described previously, UGT1A5 mRNA was not detected by duplex RT-PCR in any of the tissues examined in this study (results not shown). No differences in UGT expression were observed in RT-PCRs performed with or without primers for /H9252-actin (results not shown).

Of the family 2B UGTs (Fig. 2B), only UGT2B4 (tongue and floor of mouth) and UGT2B17 (tonsil and larynx) were detected in pooled RNA samples from human aerodigestive tract tissues by duplex RT-PCR. Although UGT2B15 and UGT2B7 were not detected in any of the aerodigestive tract tissues examined using pooled RNA samples (Fig. 2B), 2 of 10 individual esophageal specimens examined in this

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**Table 2**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>FoM</th>
<th>Tongue</th>
<th>Larynx</th>
<th>Esophagus</th>
<th>Lung</th>
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<tr>
<td>9-OH-BaP</td>
<td>2.0</td>
<td>0.74</td>
<td>5.3</td>
<td>8.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>7-OH-BaP</td>
<td>4.5</td>
<td>2.6</td>
<td>7.2</td>
<td>16.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>3-OH-BaP</td>
<td>2.6</td>
<td>1.6</td>
<td>3.9</td>
<td>10.0</td>
<td>0.032</td>
</tr>
<tr>
<td>BaP-7,8-dihydrodiol</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

FoM, floor of mouth.

'a' Rate of glucuronidation calculated based upon one experiment.

'b' Rate of glucuronidation represented was the average of two independent experiments.

'c' N.D., not detectable in assays using 0.1 mg of microsomal protein.

'd' N.D., not detectable in assays using 1 mg of microsomal protein.
study exhibited UGT2B17 expression; one esophageal specimen exhibited UGT2B15 expression (results not shown). As shown in previous studies (Beaulieu et al., 1997; Ulrich et al., 1997; King et al., 1999; Levesque et al., 1999), family 2B UGTs were well expressed in human liver. Other than UGT2B15 expression in a single lung specimen, none of the other family 2B UGTs were detected in human lung, whether by analysis of pooled or individual RNA samples.

To compare the levels of tissue expression for UGTs that were expressed in multiple aerodigestive tract tissues, semiquantitative duplex RT-PCR was performed. As shown for UGT1A10 in larynx (Fig. 3, A and B), linear increases in RT-PCR product were obtained by semiquantitative analysis, a pattern observed for all semiquantitative duplex RT-PCR analysis of UGT expression performed in this study. Using UGT/β-actin ratios as a measurement of the relative level of UGT expression, both UGT1A7 and UGT1A10 were shown to be relatively well expressed in all aerodigestive tract tissues examined (Fig. 3C). Although UGT1A6 was expressed at high levels in larynx (at levels similar to that observed for liver), low levels of expression were observed for other aerodigestive tract tissues, including esophagus. Similarly, the levels of expression of both UGTs 2B4 and 2B17 were significantly higher in liver than that observed for the UGT family 2B-expressing aerodigestive tract tissues.

Activities of Aerodigestive Tract-Expressing UGTs against 7-OH-BaP. Previous studies have implicated several UGTs in the glucuronidation of BaP metabolites (Jin et al., 1993a,b; Grove et al., 1997; Mojarrabi and Mackenzie, 1998; Strassburg et al., 1999; Guillemette et al., 2000). To better assess the relative activities of aerodigestive tract-expressing UGTs against BaP metabolites, we performed a comprehensive screening of aerodigestive tract-expressing UGT-overexpressing cell lines or baculosomes for BaP metabolite-glucuronidating activity using 7-OH-BaP as substrate. UGT1A7- and UGT1A10-overexpressing baculosomes, as well as UGT1A8-overexpressing cell homogenates, exhibited detectable levels of glucuronidating activity against 7-OH-BaP using as little as 0.1 mg of baculosome or cell homogenate protein in glucuronidation assays (Table 3). The relative affinity of each of these UGTs for 7-OH-BaP as determined by kinetic ($K_m$) analysis was $1A10 > 1A7 > 1A8$. No detectable activity against 7-OH-BaP was observed for UGTs 1A6, 2B4, or 2B17 using up to 5 mg of cell homogenate in glucuronidation assays; all were active against 1-naphthol (UGT1A6), clofibrate acid (UGT2B4), or androsterone (UGT2B17) as test substrates (results not shown).

**Discussion**

Glucuronidation has been implicated as a major detoxification pathway for many carcinogens, including polycyclic aromatic hydrocarbons like BaP and other tobacco carcinogens like NNK (Richie et al., 1997; Strassburg et al., 1999; Ren et al., 2000). Although recent studies have suggested that many enzymes within the human UGT superfamily are extra-hepatic, however, previous studies examining UGT enzyme expression patterns have focused primarily on sites not known as primary targets for tobacco-induced carcinogenesis, including the digestive tract, the prostate, and the brain (Cheng et al., 1999; Hum et al., 1999; King et al., 1999; Strassburg et al., 2000; Tukey and Strassburg, 2000). In the present study, all of the aerodigestive tract tissues examined exhibited glucuronidating activity against multiple BaP metabolites. This is consistent with the fact that several UGTs were expressed in all aerodigestive tract tissues examined in this study. Previous studies have shown that several UGTs, including UGT2B7, UGT1A7, UGT1A8, UGT1A9, and UGT1A10, are active against several BaP phenols (Jin et al., 1993a,b; Grove et al., 1997; Mojarrabi and Mackenzie, 1998; Strassburg et al., 1999; Guillemette et al., 2000). Of these, UGT1A7 and UGT1A10 were well expressed in all aerodigestive tract tissues examined in the present study, whereas UGT1A8 was expressed in larynx. In addition, all three of these UGTs exhibited activity against 7-OH-BaP, with UGT1A10 exhibiting the highest affinity for 7-OH-BaP as determined by kinetic analysis. None of the other aerodigestive tract-expressing UGTs...
(UGT1A6, UGT2B4, and UGT2B17) exhibited activity against 7-OH-BaP. These data are consistent with results from preliminary studies in our laboratory suggesting that UGT1A7, UGT1A8, and UGT1A10 all exhibit significant activity against trans-BaP-7,8-dihydropyrene, a direct precursor of the highly carcinogenic BaP-7,8-diol-9,10-epoxide (Fang et al., 2002). Together, these data suggest that UGT1A7, UGT1A8, and particularly UGT1A10 play an important role in tobacco carcinogen detoxification in the aerodigestive tract.

Differences were observed for aerodigestive tract tissues in their glucuronidating activity against different BaP metabolites in the present study. This may be due in part to differences in levels of expression of BaP metabolite-glucuronidating UGTs. For example, unlike that observed for microsomes from other aerodigestive tract tissues, laryngeal microsomes exhibited higher activity against 9-OH-BaP than 3-OH-BaP. This may be a result of the fact that in addition to UGT1A7 and UGT1A10, which were expressed in all aerodigestive tract tissues examined, UGT1A8 was also expressed in larynx.

The detection of glucuronidating activity in aerodigestive tract tissues and that specific UGTs are expressed in these tissues are consistent with recent data demonstrating that such UGTs could play an important role in tobacco-related cancer risk. Zheng et al. (2001) has demonstrated that UGT1A7 allelic variants coding for variant UGT1A7 isoforms with decreased activity against BaP phenols significantly contribute to increased risk for orolaryngeal cancer, an association that was linked to smoking. Studies are currently underway examining whether such associations may also be present for other aerodigestive tract tissue-expressing, BaP phenol-metabolizing UGTs (i.e., UGT1A10 and UGT1A8). Preliminary studies have shown that there exist at least three independent amino acid-altering polymorphisms present in the coding region of the UGT1A10 gene (Z. Zheng and P. Lazarus, unpublished results). The presence of these polymorphisms may be particularly important in risk assessment studies of aerodigestive tract cancer risk given the activity of UGT1A10 toward BaP metabolites. The functional significance of these polymorphisms and their potential role in aerodigestive tract cancer risk is currently being assessed.

The data presented in this study strongly suggest that glucuronidation is not a major metabolic pathway/detoxification mechanism in human lung. Similar to that observed in other studies of other substrates, including the NNK metabolite 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol and 4-nitrophenol (Ren et al., 2000), no glucuronidating activity was observed in microsomes from human lung specimens against any BaP metabolite tested. Analysis was performed using up to 1 mg of lung microsomal protein and was performed for three normal lung specimens from three individual subjects. These data are consistent with the fact that none of the UGTs screened in this study were expressed in lung tissue, as determined by duplex RT-PCR of pooled RNA samples. These data are consistent with previous studies of UGT expression in lung. Using duplex RT-PCR assays similar to that described in the present study, no expression of UGT1A9 and, at best, low levels of expression of UGT2B7 were detected by Ren et al. (2000), whereas King et al. (1999) showed that UGT1A6 and UGT2B7 were not expressed in lung. This is in contrast to that observed by Hum et al. (1999), who suggested that all family 2B UGT enzymes are expressed in human lung tissue. The reason for the disparity observed between this latter study and other studies remains unclear.

In the present study, only UGT1A6, UGT1A7, and UGT1A10 were detected in RNA samples from esophageal tissues from individual subjects. This contrasts with previous results from Strassburg et al. (1999) who, in addition to UGT1A7 and UGT1A10, found that UGTs 1A8, 1A9, and 2B7 were also expressed in two human esophageal specimens and that UGT2B15 was detected in one of two specimens. In addition, contrary to that observed in the present study, no esophageal UGT1A6 expression was detected in previous studies (Strassburg et al., 1999). These discrepancies could be due to several potential factors, including site of specimen collection (i.e., upper versus lower esophagus), polymorphic expression of individual UGT enzymes, or effects on UGT inducibility by exogenous exposures. Previous studies have indicated that, based upon RT-PCR analysis, certain UGTs exhibit polymorphic expression (Strassburg et al., 1998a, 2000). Multiple UGTs were shown to exhibit differential expression in normal gastric (Strassburg et al., 1998a), duodenum, jejunum, ileum (Strassburg et al., 2000), and esophagus (Strassburg et al., 1999). Although UGT2B15 and UGT2B17 expression was not detected by analysis of pooled samples in the present study, expression of UGT2B17 was detected in esophageal tissue for 2 of 10 subjects when RNA samples were analyzed individually, whereas UGT2B15 was detected in one esophageal and one lung specimen. These data support results from previous studies suggesting that certain UGTs are either inducible or may exhibit polymorphic expression.

In summary, the data presented in this study demonstrate that aerodigestive tract tissues exhibit significant glucuronidating activity against BaP metabolites and that UGT enzymes with activity against these metabolites are expressed in aerodigestive tract tissues. These results are consistent with recent studies suggesting that specific UGTs play an important role in the detoxification of tobacco carcinogens and in risk for aerodigestive tract cancer (Zheng et al., 2001). Further studies are currently being performed examining whether BaP metabolite-glucuronidating UGTs may play a similar role for other cancers of the digestive tract (i.e., colon) in which BaP exposure is also etiologically important.

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References


