Expression of Glutathione-Dependent Enzymes and Cytochrome P450s in Freshly Isolated and Primary Cultures of Proximal Tubular Cells from Human Kidney

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ABSTRACT

The expression of glutathione (GSH)-dependent enzymes and cytochrome P450 (P450) proteins in freshly isolated proximal tubular cells from human kidney (hPT), and the effect of primary culture on these enzymes, were determined. Freshly isolated hPT cells had relatively high activities of γ-glutamyltransferase, γ-glutamylcysteine synthetase, glutathione S-transferase (GST), glutathione disulfide reductase, and GSH peroxidase. Cytochrome P450 4A11 was detected in freshly isolated hPT cells, whereas CYP2E1 was not. Freshly isolated hPT cells also expressed GSTA, GSTP, and GSTT but not GSTM. Primary cultures of hPT cells maintained their epithelial-like nature and diploid status, based on measurements of morphology, cytokeratin expression, and flow cytometric analysis. hPT cells retained GSH-dependent enzyme activities during primary culture, whereas cells that had undergone subsequent passage exhibited a loss of activities of most GSH-dependent enzymes and no longer expressed P450s or GSTs. CYP4A11 expression in primary cultures of hPT cells was significantly increased after treatment for 48 h with either ethanol (50 mM) or dexamethasone (7 nM). GSTA, GSTP, and GSTT contents, although still detectable, were decreased compared with those of freshly isolated hPT cells. Our data show that hPT cells express enzymes involved in xenobiotic disposition, and that they thus provide a model suitable for studies of human renal drug metabolism. Furthermore, primary cultures of hPT cells may afford the opportunity to study factors regulating P450 enzyme expression in human kidney.

The mammalian kidney is a complex organ composed of numerous different cell types that function together to facilitate the filtering of blood and the regulation of systemic blood pressure (Guyton, 1991; Tisher and Madsen, 1996). The mammalian kidney also can oxidize and conjugate drugs because xenobiotic-metabolizing enzyme expression and/or activity have been reported in this tissue (for review, see Lohr et al., 1998). Rat and rabbit kidneys have been used for a number of years in both physiological and toxicological studies (Detrisac et al., 1984; Tri- fillis et al., 1985; Kempson et al., 1989; Chen et al., 1990; Rodilla et al., 1998), and primary cultures of human kidney cells originating mainly from the proximal tubules have been used for a number of years in both physiological and toxicological studies (Detrisac et al., 1984; Tri- fillis et al., 1985; Kempson et al., 1989; Chen et al., 1990; Lash et al., 1995; Cummings et al., 1997).

There have been several reports on select aspects of renal drug metabolism in humans (Hayes and Pulford, 1995; Amet et al., 1997; Rodilla et al., 1998), and primary cultures of human kidney cells originating mainly from the proximal tubules have been used for a number of years in both physiological and toxicological studies (Detrisac et al., 1984; Tri- fillis et al., 1985; Kempson et al., 1989; Chen et al., 1990; Lash et al., 1995; Cummings et al., 1997).

Abbreviations: P450, cytochrome P450; GST, glutathione S-transferase; GSH, glutathione; hPT, human proximal tubular; GPX, glutathione peroxidase; GRD, glutathione disulfide reductase; GCS, γ-glutamylcysteine synthetase; GGT, γ-glutamyltransferase; PAGE, polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate.

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3 The standard nomenclature for P450 isoenzymes described in Nelson et al. (1996) is used in this study.

ABBREVIATIONS: P450, cytochrome P450; GST, glutathione S-transferase; GSH, glutathione; hPT, human proximal tubular; GPX, glutathione peroxidase; GRD, glutathione disulfide reductase; GCS, γ-glutamylcysteine synthetase; GGT, γ-glutamyltransferase; PAGE, polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate.
Garrett et al., 1998). However, the composition of P450 hepatoproteins and glutathione (GSH)-dependent enzymes in the human kidney, freshly isolated human proximal tubular (hPT) cells, and/or cultures of hPT cells have been poorly characterized. A description of the drug-metabolizing properties of human kidney cells could provide not only a better explanation of their biochemical and physiological responses to xenobiotics but also could decrease the uncertainty involved in extrapolating such data obtained in animals to humans. Development of an in vitro model that mimics human kidney cell function in vivo would have obvious benefits in pharmacology and the study of toxicological risk assessment. Although cell lines are available and human kidney cells can be immortalized (Taub, 1996), these various models use transformed cell lines that, at least with regard to drug metabolism, may no longer be suitable for comparison to the in vivo state.

The goals of this study were to determine the expression of exemplary drug-metabolizing enzymes in hPT cells isolated from kidney samples and to assess the effects of primary culture on these enzymes. Freshly isolated hPT cells were found to express high levels of certain P450s and GSH-dependent enzymes. Although primary hPT cell cultures exhibited a decrease in activity and/or content of most of these enzymes, enhanced expression of one enzyme, namely CYP4A11, was observed in cultures exposed to ethanol and/or dexamethasone. Our data indicate that hPT cells can serve as a suitable model for studies of human renal drug metabolism, and that primary cultures of these cells can be used to study factors regulating renal P450 enzyme expression.

Materials and Methods

Chemicals. Unless otherwise stated, all chemicals used were purchased from Sigma Chemical Co. (St Louis, MO). 1-[14C]Laureic acid (specific activity = 40 mCi/mmol) was purchased from ICN Pharmaceuticals, Inc. (Irvine, CA) and exhibited radiochemical purity >99%, as assessed by thin-layer chromatography. Antibodies for CYP2E1, GSTA, GSTP, and GSTM were purchased from Oxford Biomedical (Oxford, MD). The antibody for GSTT was purchased from Biorin International (Newton, MA). Note that the convention used for naming GST isoforms is that Greek letters are used for rat enzymes, whereas Arabic letters are used for the analogous human enzymes.

The CYP2E1 antibody was a rabbit polyclonal anti-human CYP2E1, prepared against recombinant human liver CYP2E1. GST antibodies used were as follows: GSTA antibody is a polyclonal goat anti-rat GST Ya antibody, prepared against affinity-purified rat liver GST Ya, is specific for rat and human GSTa(A), and cross-reacts with neither GSTm(M) nor GSTπ(P) class isozymes; GSTP antibody is a polyclonal rabbit anti-human GST P1-1 antibody, prepared against recombinant human GST P1-1, that was expressed in Escherichia coli, is specific for rat and human GSTπ(P), and cross-reacts with neither GSTm(M) nor GSTa(A) class isozymes; GSTM antibody is a polyclonal goat anti-rat GST Yb antibody, prepared against affinity-purified rat liver GST Yb1, is specific for rat and human GSTm(M), and cross-reacts with neither GSTa(A) nor GSTπ(P) class isozymes; and GSTT antibody is a polyclonal rabbit anti-human GSTT antibody, prepared against purified human liver GSTT2, is specific for rat and human GSTT(π), and cross-reacts with neither GSTa(A), GSTπ(P), nor GSTm(M) class isozymes.

Isolation of Microsomes and Cytosol from Human Kidney Homogenates. Freshly isolated slices of human kidney cortex were obtained from the Human Tissue Resources Core of the Department of Pathology, Harper Hospital (Detroit, MI). Human kidney samples were obtained from 15 female (age = 60.9 ± 11.8; mean ± S.D.; range = 45–77) and 11 male (age = 63.6 ± 9.5; mean ± S.D.; range = 44–77) patients, each of whom underwent a unilateral nephrectomy. Each slice (generally 1–4 g of tissue) was weighed, rinsed with buffer (250 mM sucrose, 10 mM triethanolamine/HC1, 1 mM EDTA-Na2, pH 7.6), and homogenized in 3 ml of buffer/g tissue with a Teflon-gllass device. Homogenates were initially centrifuged at 9000 g for 20 min. The supernatant was filtered through cheese cloth and centrifuged for 60 min at 105,000 g. The resulting supernatant (cytosolic fraction) was used for enzyme assays and was stored at −80°C until use. The resulting pellets were resuspended in buffer and centrifuged an additional 60 min at 105,000 g to produce “washed” microsomes. The microsomal pellets were resuspended in buffer containing 10% (v/v) glycerol and were stored at −80°C until used. Enzyme activities were normalized to protein concentrations, which were measured with the BCA protein kit from Sigma Chemical Co. according to the manufacturer’s instructions.

Isolation of hPT Cells from Renal Cortical Slices. hPT cells were derived from human kidney cortical slices obtained from the source described above after scoring by a pathologist as normal (i.e., derived from noncancerous, nondiseased tissue). The fibrous renal capsule was removed from the slice, and the slice was then weighed. The slices were washed with sterile PBS, minced, and the pieces were placed in a trypsinization flask filled with 30 ml of Hanks’ buffer containing 25 mM NaHCO3, 25 mM HEPES, pH 7.4; 0.5 mM EGTA; 0.2% (w/v) BSA; 50 μg/ml gentamicin; 1.3 mg/ml collagenase; and 0.59 mg/ml CaCl2, which was filtered before use. All buffers were continuously bubbled with 95% O2, 5% CO2 and were maintained at 37°C. Minced cortical pieces were subjected to collagenase digestion for 15 min, after which the supernatant was filtered through a 70-μm mesh filter to remove tissue fragments, centrifuged at 150g for 7 min, and the pellet resuspended in Krebs-Henseleit buffer I (118 mM NaCl, 4.8 mM KCl, 0.96 mM KH2PO4, 0.12 mM MgSO4 · 7H2O, 25 mM NaHCO3, 25 mM HEPES, and 2% BSA (w/v)). These steps were repeated until complete digestion of the tissue was achieved (usually four or five cycles). Resuspended cells were combined and centrifuged at 150g for 7 min, pellets were washed with Krebs-Henseleit buffer I, centrifuged at 150g for 7 min, and the final pellet (hPT cells) was resuspended in Krebs-Henseleit Buffer II (same as Krebs-Henseleit buffer I except no BSA was added). Approximately 50 to 70 × 106 cells were obtained from 1 g of human kidney cortical tissue.

Culturing of hPT Cells. Isolation of hPT cells was achieved as explained above, except sterile conditions were used (i.e., all instruments and glassware were autoclaved and all buffers were filtered through a 0.2-μm pore-size filter). After isolation, cells were resuspended in 2 ml of Krebs-Henseleit Buffer II and diluted to 30 ml with cell culture media. Basal medium was a 1:1 mixture of Dulbecco’s modified Eagle’s medium:Ham’s F12. Standard supplementation included 15 mM HEPES, pH 7.4; 20 mM NaHCO3; antibiotics for day 0 through day 3 only (192 I.U. penicillin G/ml + 200 μg of streptomycin sulfate/ml) to inhibit bacterial growth; 2.5 μg of amphotericin B/ml to inhibit fungal growth; 5 μg of bovine insulin/ml (0.87 μM); 5 μg of human transferrin/ml (0.66 mM); 30 mM sodium selenite; 100 ng of hydrocortisone/ml (0.28 μM); 100 ng of epidermal growth factor/ml (17 nM); and 7.5 pg of 3,3’,5-triiodo- DL-thyronine/ml (111 nM) (Lasch et al., 1995). Cells were seeded at densities of 50 to 100 μg of protein/cm2 (0.5–1.0 × 105 cells/ml) in polystyrene culture dishes. Cultures were grown at 37°C in a humidified incubator under an atmosphere of 95% air, 5% CO2 at pH 7.4. Cultures were allowed to attach and grow for at least 24 h before treatment with any agent. Cells were harvested from the dishes by either scraping the plates with a Teflon scraper or by brief incubation with 0.05% (w/v) trypsin/0.2% (w/v) EDTA (in Ca2+- and Mg2+-free Hanks’ buffer).

Isolation of Microsomes and Cytosol from hPT Cells. Microsomal and cytosolic fractions were prepared from both freshly isolated and primary cultures of hPT cells by homogenization of the
cells with a Polytron ultrasonic device (Brinkmann Instruments, Westbury, NY), followed by centrifugation at 11,000g for 20 min to pellet nuclei, mitochondria, and cellular debris. The supernatant from this step was centrifuged in a tabletop ultracentrifuge at 105,000g for 90 min at 4°C to separate the cytosolic fraction from the microsomal pellet.

**Enzyme Assays.** Activities of glutathione peroxidase (GPX) with 0.25 mM H₂O₂ as substrate; GST with 1-chloro-2,4-dinitrobenzene as substrate; glutathione disulfide reductase (GRD), γ-glutamylcysteine synthetase (GCS), and γ-glutamyltransferase (GGT) with γ-glutamyl-p-nitroaniline and glycylglycine as substrates; and hexokinase were determined by spectrophotometric assays as described previously (Lash and Tokarz, 1989; Lash et al., 1998 and references therein). Total extracts of cells were used as the source of enzymes for each assay.

Lauric acid ω- and ω-1 hydroxylation, the former of which is catalyzed specifically by CYP4A11 (Powell et al., 1996), were determined as described by Miranda et al. (1990) and Salhab et al. (1987). Incubation mixtures (0.5 ml) contained 50 mM Tris-HCl buffer, 0.5 to 1.0 mg of microsomal protein from human kidney, 1 mM NADPH, and 100 μM [14C]lauric acid. The reactions were initiated with NADPH and terminated after 15 or 30 min at 37°C with 100 μl of 10% (v/v) H₂SO₄. After adding 5 μl of 1 mM tobutamide as an internal standard, lauric acid and its metabolites were isolated from the reaction mixtures by extraction into 3 ml of diethyl ether. The internal standard, lauric acid and its metabolites were resolved from their 12-hydroxy and 11-hydroxy metabolites by isocratic elution with methanol/H₂O/acetic acid (62:37.8:0.2) with a flow rate of 0.22 ml/min. Lauric acid gave retention times of 8 min, 16 to 20 min, and 48 min, respectively. Under these HPLC conditions, 12-hydroxylauric acid, 11-hydroxylauric acid, and lauric acid were detected least significant differences between means for data were first assessed by a one-way ANOVA. When significant F values were obtained, the Fisher’s protected least significance t test was performed to determine which means were significantly different from one another, with two-tail probabilities < .05 considered significant.

**Results**

**Morphology and Expression of Cytokeratins and Vimentin in Primary Cultures of hPT Cells.** Primary cultures of hPT cells grown for 4 days, at which time the cultures reach or approach confluence, exhibited characteristic epithelial morphology (Fig. 1A). Expression of cytokeratins, a marker for epithelial cells, and of vimentin, a marker for endothelial cells, was assessed in two separate confluent primary cultures of hPT cells by immunohistochemical staining with monoclonal FITC-conjugated mouse antibody to cytokeratins and a monoclonal Texas Red conjugated vimentin antibody to vimentin. Primary cultures of hPT cells expressed high levels of cytokeratins after 4 days of cell culture (Fig. 1B). In contrast, vimentin staining was not detected in these cells (data not shown).

**Flow Cytometry Analysis of Primary Cultures of hPT Cells.** Flow cytometry analysis of primary cultures of hPT cells was performed to assess the proportion of these cells in different phases of the cell cycle (Fig. 2). This method also can detect cells undergoing apoptosis, which would appear to the left of the G₂/G₁ peak in the subdiploid region. The confluent hPT cells (4 days of cell culture) were all diploid, viable, and predominantly in the G₂/G₁ phase of the cell cycle, with <10% of the cells in the S phase and no apoptotic cells.

**Activities of GSH-Dependent Enzymes in Freshly Isolated hPT Cells.** Activities of GGT, GCS, GRD, GPX, and hexokinase were measured in freshly isolated hPT cells from three individual donors (Fig. 3). Viability of these cells after isolation was typically ~90%, as determined by trypan blue exclusion and lactate dehydrogenase release (data not shown). Freshly isolated hPT cells had ~10 times higher levels of GGT, a proximal tubular cell marker enzyme on the brush-border membrane, than hexokinase, a distal tubular cell marker enzyme, consistent with these cells being derived from the proximal tubular region of the nephron (Lash and Tokarz, 1989). Specific activities of the other four enzymes; glutathione peroxidase (GPX) with 0.25 mM H₂O₂ as substrate; glutathione disulfide reductase (GRD), γ-glutamylcysteine synthetase (GCS), and γ-glutamyltransferase (GGT) with γ-glutamyl-p-nitroaniline and glycylglycine as substrates; and hexokinase were determined by spectrophotometric assays as described previously (Lash and Tokarz, 1989; Lash et al., 1998 and references therein). Total extracts of cells were used as the source of enzymes for each assay.

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A. Primary Cultured hPT Cells

GSH-dependent enzymes varied somewhat between individual donors, but were all within a factor of 5 of each other.

Expression of CYP4A11 and CYP2E1 in Freshly Isolated hPT Cells. Freshly isolated hPT cells from five male and three female patients ranging in age from 44 to 77 years were analyzed for expression of CYP4A11 with a polyclonal rabbit anti-human antibody (Fig. 4, A and B). All of the patients tested expressed CYP4A11 and there was no major difference in expression among any of the patients tested, based on densitometric analysis.

CYP2E1 expression was demonstrated by measurement of v-hydroxylauric acid hydroxylation with HPLC analysis and radiochemical detection. In microsomes prepared from homogenates of human renal cortical slices, GRD activity significantly increased in passage 1 but decreased significantly in passages 2 to 4. The activities of GSTA and GSTT increased significantly in passages 2 to 4. The activities of GSTA and GSTT were analyzed for expression of GSTA with a polyclonal goat anti-human GSTA antibody. GSTA was detected in all patients tested (Fig. 5E) and levels did not vary significantly among the patients tested (Fig. 5F).

Effect of Duration of Cell Culture on Activity of CYP4A11 in hPT Cells. Freshly isolated hPT cells were seeded at a density of 0.5 to 1.0 × 10^6 cells/ml in the supplemented cell culture media. Cells were grown to confluency (~4 to 5 days) and isolated from culture dishes by trypsin-EDTA treatment. At each passage, one-half the cells was harvested and the other half was subcultured. Figure 6 displays the activity of GSH-dependent enzymes and hexokinase in freshly isolated hPT cells (passage 0) and after four subsequent passages. The ratio of GGT to hexokinase decreased slightly compared with that in freshly isolated cells, but GGT activity was still 5 times higher than hexokinase activity at passage 4. GGT activity remained relatively constant through passage 4. GRD activity significantly increased in passage 1 but decreased significantly in passages 2 to 4. The activities of GST and GPX remained unchanged until passage 2, after which significant decreases were observed. GCS activity remained unchanged throughout the four passages.

B. Cytokeratin Staining

Fig. 1. Photomicrograph and expression of cytokeratins in primary cultures of hPT cells. Freshly isolated hPT cells were seeded at a density of 0.5 to 1.0 × 10^6 cells/ml and allowed to grow to confluency (~4 to 5 days). Cells were incubated with a monoclonal FITC-conjugated mouse cytokeratin antibody and cytokeratin staining was visualized with laser scanning microscopy at 340 nm. Original magnification, 100×.
slightly higher than those in freshly isolated hPT cells. This was not due to loading differences because equal amounts of protein were loaded onto the gel.

The effect of solvent alone (i.e., ethanol) on CYP4A11 expression in primary cultures of hPT cells was assessed (Fig. 8A). In the absence of any solvent in the media, the expression of CYP4A11 in cells cultured for 72 h decreased to ~50% of that in freshly isolated cells (data not shown). Treatment of primary cultures of hPT cells with 25 mM ethanol had no effect on CYP4A11 expression, whereas treatment of cells with 50 mM ethanol produced a slight increase in CYP4A11 expression compared with control cells.
We then examined the effect of dexamethasone (a known inducer of hepatic CYP3A isoforms and generally stabilizes P450 expression in hepatocyte cultures) in the presence and absence of ethanol on CYP4A11 expression in primary cultures of hPT cells (Fig. 8B). Again, without the addition of solvent, the expression of CYP4A11 decreased to 25% of that in freshly isolated cells. Dexamethasone (7 nM) increased CYP4A11 expression over control after 48 h of treatment. Ethanol (50 mM) treatment also resulted in a slight increase in CYP4A11 expression compared with control. Cells treated with both ethanol and dexamethasone had significantly higher levels of CYP4A11 expression than control cells but equal levels of CYP4A11 expression compared with cells treated with ethanol or dexamethasone alone.

Expression of GSTA, GSTP, and GSTT in Primary Cultures of hPT Cells. Freshly isolated hPT cells were seeded at a density of 0.5 to 1.0 × 10^6 cells/ml and allowed to grow to confluency (~4 to 5 days). Cells were then harvested,
cytosol was isolated, and expression of GSTA, GSTP, and GSTT determined by immunoblot analysis. GSTA was detected in 30 μg of cytosolic protein isolated from six separate cultures, representing two different patients, after 4 days of culture [Fig. 9A, sample 98-415 (lanes 2–4) and sample 99-026 (lanes 5–7)]. Although a direct comparison of expression in freshly isolated cells and cell cultures is not possible due to different amounts of protein loading, GSTA expression appeared to be well maintained during the course of primary culture (Fig. 9A, lane 1). The expression of GSTP and GSTT in cytosol isolated from hPT cells after 4 days of culture was determined by Western blot analysis [Fig. 9A, B (lanes 2–4) and C (lanes 2–5)]. Unlike GSTA, the expression of both GSTP and GSTT appeared to decrease significantly from levels seen in freshly isolated hPT cells (Fig. 9A, B and C, lane 1).

Discussion

The present results describe the use of freshly isolated and primary cultures of hPT cells as models to study drug metabolism mediated by P450 and GSH-dependent enzymes in the human kidney. Confluent primary cultures of hPT cells maintained their epithelial properties, as demonstrated by cytokeratin and vimentin expression, and remained viable and diploid, as shown by flow cytometry. Freshly isolated hPT cells exhibited high levels of GSH-dependent enzyme activities, and subcellular fractions prepared from these cells expressed specific P450 and GST isoforms. We showed that GSTT is expressed in human kidney and that CYP4A11 is present in the proximal tubules. In other studies (Lasker et al., 2000), we found renal CYP4A11 to be localized exclusively in the S2 and S3 segments of the proximal tubules. hPT cells retained GSH-dependent enzyme activities during primary culture, whereas cells that had undergone subsequent passage exhibited a loss of most GSH-dependent enzyme activities and no longer expressed P450s or GSTs. Nevertheless, steady-state levels of CYP4A11 in hPT cell primary cultures could be enhanced by treatment with specific xenobiotics. Primary cultures of hPT cells thus appear to be a suitable in vitro model for studying regulation of the renal expression of CYP4A11 and specific GST isoforms.

Activity and Expression of Renal P450 Enzymes

In contrast to human liver, human kidney appears to express few P450 enzymes. In fact, the only P450 enzymes found at significant levels in human renal microsomes are CYP4A11 and CYP4F2 (Lasker et al., 2000) and CYP3A isoforms (Schuetz et al., 1992; Kharasch et al., 1995). Herein, we also were able to detect CYP4A11 but not CYP2E1 in microsomes from freshly isolated hPT cells. This agrees with a study by Amet et al. (1997), which reported the presence of a single “CYP4A” immunoreactive protein in human kidney microsomes with a polyclonal sheep anti-rat CYP4A1 antibody and that CYP2E1 was undetectable in renal microsomes from 18 different subjects. The catalytic activity of renal CYP4A11, as measured by lauric acid hydroxylation, was significantly lower than the mean value reported by Amet et al. (1997). However, the lauric acid ω-hydroxylase activities reported by
Amet et al. (1997) varied by >10-fold and the activities reported herein are at the lower end of that range. Differences in assay methods also may have contributed to these differences in rates.

**Activity and Expression of GSH-Dependent Enzymes.** Freshly isolated hPT cells expressed high levels of several GSH-dependent enzymes. This report describes activities of enzymes that are critical to second-phase drug metabolism and redox status in hPT cells. Certain of these GSH-dependent enzymes (GCS, GPX, and GRD) exhibited activities that were higher than those measured in rat kidney PT cells, whereas other enzyme activities (GST, GGT, and hexokinase) were lower (Lash et al., 1995). Whereas the overall activity of GST (with 1-chloro-2,4-dinitrobenzene as substrate) was lower in hPT cells than in rat kidney PT cells, GSTA, GSTP, and GSTT were expressed in hPT cells, whereas only GSTα and GSTμ could be detected in rat kidney cells (Fig. 5; Cummings et al., 2000). Such interspecies differences in GST activity and in GST isoform expression may be explained by the markedly different affinities of the various GST isoforms for 1-chloro-2,4-dinitrobenzene (Manner-vik, 1985).

The expression of GSTA and GSTP but not of GSTM in freshly isolated hPT cells is in agreement with previous reports (Terrier et al., 1990; Campbell et al., 1991; Hiley et al., 1994; Rodilla et al., 1998). These other studies also showed low levels of renal GSTM expression in some patients, especially when the other two isoforms were absent. It is not surprising that we failed to detect renal GSTM expression in the present study because a genetic polymorphism exists that is characterized by 50 to 60% of the human population not expressing this isoform (Hayes and Pulford, 1995). Renal GSTM expression also is increased in subjects exhibiting kidney neoplasias and/or tumor growth (Rodilla et al., 1998). We observed no apparent sex-dependent differences in the expression of the various GST isoforms, although the sample size was too small to draw any definitive conclusions. Despite this small subject population, the extensive variation noted in renal GSTP expression suggests that a polymorphism may exist. To the best of our knowledge, this is also the first time that GSTT expression in the human kidney has been reported.

**Comparisons of P450 and GST Isoform Expression between Rat and Human Kidney.** From the data presented herein and our previous work (Cummings et al., 1999, 2000), it is obvious that rat and human kidneys differ significantly in the expression of GST and P450 enzymes. This information bears directly on the utility of data from rat studies for extrapolation to humans for risk assessment. For example, chemicals such as acetaminophen, various chloroethylenes, and other low-molecular-weight hydrocarbons are metabolized by CYP2E1 (Guengerich, 1991). Although these compounds are metabolized primarily in the liver, it is possible, especially in cases of exposure to high chemical concentrations (e.g., suicide attempts), that the kidneys also are exposed to significant levels of these compounds. In such cases, the differences observed in renal CYP2E1 expression between rats and humans may play a pivotal role in the development of nephrotoxicity. Although the majority of studies on xenobiotic metabolism and kidney toxicity have been performed in rats, the results obtained with nephrotoxins activated by CYP2E1 would not be applicable to humans due to the lack of expression of this P450 enzyme in the human kidney. However, both rat and human kidney exhibit extensive expression of CYP4A enzymes as well as substantial lauric acid ω-hydroxylase activity. However, the rat kidney may only be somewhat applicable for the study of renal CYP4A function and/or expression in humans because four CYP4A subfamily proteins are found in the rat kidney (CYP4A1, CYP4A2, CYP4A3, and CYP4A8) (Ito et al., 1998; Nguyen et al., 1999), whereas the human kidney expresses only a single CYP4A enzyme, namely, CYP4A11 (Powell et al., 1996, 1998).

**Influence of Primary Culture and Subculturing on Enzyme Expression and Cellular Function.** Much of the previous work with primary cultures of hPT cells has focused on nondrug metabolism issues. Data from this study describe for the first time changes that occur in the drug-metabolizing capacity of primary and subcultures of hPT cells. The cyto-keratin and vimentin expression data and the flow cytometry analysis presented herein indicate that the confluent, primary cultures of hPT cells are normal, diploid epithelial cells. As would be expected for hPT cells in the intact kidney, primary cultures of hPT cells are mostly in the resting phase and are not undergoing cell death by either necrosis or apoptosis. Thus, these cells may provide a useful tool for studying the injurious effects of chemicals on the hPT cell cycle. Importantly, activities of GSH redox cycle enzymes as well as those of GSH synthesis and degradation enzymes were maintained in primary cultures of hPT cells (Fig. 6). The expression of GSTA also was retained in primary hPT cell cultures, whereas that of GSTP and GSTT decreased. Both GSTP and GSTT were still detected after 4 days of culture but at levels that were apparently much lower than those of freshly isolated hPT cells. The differences observed in GST enzyme expression in these cultures may reflect the role of each individual isoform in kidney function or may stem from differences in the manner via which expression of each isoform is regulated. Interestingly, GSH-dependent enzyme activities were generally not well maintained after subculturing. This suggests that use of the hPT cells after passage should be done with caution, particularly if the chemical being studied is metabolized by any of the enzymes whose expression changes under these conditions.

CYP4A11 expression was detected without the aid of an inducer for up to 3 days in primary culture. Furthermore, ethanol and dexamethasone increased the steady-state concentration of CYP4A11 protein. CYP4A11 expression showed modest decreases after 2 days of culture and typically disappeared after 4 days of culture. This time period coincided with the cells reaching confluency and residing predominately in the G0/G1 phase of the cell cycle. These findings are of importance for several of reasons. First, maintenance of CYP4A11 in cell culture, for even as little as 3 to 4 days, allows for the study of the mechanism(s) by which CYP4A11 expression is regulated. Second, there is a strong link between kidney disease and alcoholism (Vamvakas et al., 1998), and the dose of ethanol used in our studies (50 mM) is not much higher than blood alcohol levels found (22 mM) in individuals who are at “the legal limit for driving under the influence” in most states (Lands, 1998). Thus, the alcohol dose used herein is commonly encountered in the human population. The effect of dexamethasone on CYP4A11 expression suggests that hormonal regulation involving a glucocor-
Expression of Drug-Metabolizing Enzymes in Human Kidney Cells

Summary and Conclusions

The expression and activity of several drug-metabolizing enzymes were determined in freshly isolated hPT cells and in primary cultures thereof. The drug-metabolizing enzymes detected in freshly isolated hPT cells were all found in primary hPT cell cultures but at lesser levels. These data provide validation for the use of primary cultures of hPT cells to study chemical-induced injury and physiological functions of the human kidney.

References


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