Drug-Metabolizing Enzymes and Transporters: Expression in the Human Prostate and Roles in Prostate Drug Disposition

Review

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Local biotransformation enzymes and transporter proteins in tissues may exert a profound effect on drug pharmacokinetics in those tissues. Thus, the use of drugs for the treatment of benign prostatic hyperplasia (BPH) and cancer of the prostate may be influenced by high level expression of cytochrome P450 (CYP) phase I and phase II conjugation enzymes and drug transporters. Phase I drug-metabolizing enzymes detected in the human prostate include CYP, CYP1A1, CYP1A2, CYP1B1, CYP2C19, CYP2D6, CYP3A5, and CYP4B1 in both normal and tumorous tissue; CYP1A1, CYP1A2, and CYP1B1 in BPH tissues; and CYP1A1 and CYP1A2 in prostate cancer cell lines and normal prostate epithelial cells. Epoxide hydrolase was also found in prostate tumor and nonneoplastic tissue. Phase II metabolizing enzymes detected in the prostate were glutathione S-transferases (GSTs) GST-α, GST-μ, and GST-π, and N-acetyltransferase (NAT) isoforms, NAT1 and NAT2. Prostate tissue contains the multidrug resistance protein (MRP) transporters MRP1, MRP2, MRP3, and MRP4, and the multidrug resistance (MDR)-1 protein (P-glycoprotein; P-gp). Metabolism of drugs used in BPH (eg, finasteride and tamsulosin) and anticancer agents have been examined in the liver or liver preparations but not in the prostate or prostate tissue/cell lines. Thus, the published data to date shows that the prostate contains the major phase I and II drug-metabolizing enzymes as well as drug transporters. Future studies should examine the ability of the prostate to metabolize drugs used in either BPH or prostate cancer and to gauge the ability of the identified drug transporters to facilitate

entry into or efflux from the prostate. Such studies could identify the principal determinants of the local (prostate) concentrations of these therapeutic agents when used in either BPH or prostate cancer.

Use of pharmacologic agents in the treatment of benign prostatic hyperplasia (BPH) and prostate cancer is well established. Refinements to therapy with existing agents and the development of safer and more effective agents are complementary approaches for the optimization of therapy. The therapeutic goals in BPH treatment are the reduction of prostate volume, minimizing risk of acute urinary retention and the prevention of BPH-related surgery and deterioration of the quality of life of the patient. In prostate cancer, drug therapy aims to inhibit the growth of androgen-dependent tumors, to prevent their progression to the hormone-independent metastatic stage, and to decrease morbidity and mortality in advanced disease.

Factors that influence intracellular drug concentrations and the duration of drug action are important determinants of success in the treatment of BPH and prostate cancer. Rates of drug absorption and elimination are controlled by biotransformation enzymes and transporter proteins. The principal site of drug biotransformation is the liver, but it is now clear that drug-metabolizing enzymes are present in extrahepatic tissues, including the prostate (Table 1), and may affect local rates of drug biotransformation. Similarly, drug transporters are widely distributed in tissues and overexpression of these genes is associated with drug resistance, especially in tumor cells, because of the inability to maintain therapeutic drug levels.

Drug Biotransformation by Phase I and Phase II Enzymes

Most drugs and other xenobiotics undergo biotransformation to products that are more water-soluble and more efficiently excreted. In phase I reactions, a functional group is introduced into the xenobiotic substrate (usually by oxidation), resulting in a modest increase in hydrophilicity. The most important phase I enzymes are the cytochromes P450 (CYPs), a superfamily of hemoproteins found in the endoplasmic reticulum of the cell. In phase II biotransformations, the phase I products are coupled enzymically to highly polar endogenous moieties to generate metabolites that are more readily excreted.

Table 1. *Expression of biotransformation enzymes and transporters in human prostate*

Gene Product	Expression Characteristic	References
Phase I		
CYP1A1	mRNA	Williams et al, 2000
CYP1A2	mRNA	Williams et al, 2000; Cui et al, 2000; Finnström et al, 2001
CYP1A	protein	Murray et al, 1995
CYP1B1	mRNA	Williams et al, 2000; Finnström et al, 2001
	protein	Muskhelishvili et al, 2001; Carnell et al, 2004
CYP2C9	mRNA	Klose et al, 1999
CYP2C19	mRNA	Finnström et al, 2001
CYP2C	protein	Murray et al, 1995; Yokose et al, 1999
CYP2D6	mRNA	Finnström et al, 2001
CYP3A4	mRNA	Finnström et al, 2001
CYP3A5	mRNA	Agundez et al, 1998; Yamakoshi et al, 1999; Finnström et al, 2001; Koch et al, 2002
CYP3A7	mRNA	Finnström et al, 2001; Koch et al, 2002
CYP3A43	mRNA	Domanski et al, 2001
CYP3A	protein	Murray et al, 1995
CYP4B1	mRNA	Finnström et al, 2001
Epoxide hydrolase	protein	Murray et al, 1995
Phase II		
N-acetyltransferases (NATs)		
NAT1	mRNA	Wang et al, 1999
NAT2	mRNA	Wang et al, 1999; Cui et al, 2000
Glutathione S-Transferases (GSTs)		
GST-α (GSTA)	protein	Tew et al, 1987; Di Ilio et al, 1990; Murray et al, 1995; Parsons et al, 2001
GST-μ (GSTM)	mRNA	Chetcuti et al, 2001
	protein	Tew et al, 1987; Di Ilio et al, 1990; Murray et al, 1995
GST-π (GSTP)	protein	Tew et al, 1987; Di Ilio et al, 1990; Sundberg et al, 1993; Murray et al, 1995; Cookson et al, 1997; Moskalul et al, 1997; Sullivan et al, 1998; Montironi et al, 1999; Montironi et al, 2000
GST-θ (GSTT)	mRNA	Elek et al, 2000
UDP-glucuronosyltransferases (UGTs)		
UGT1A9	protein	Albert et al, 1999
UGT2B15	mRNA	Belanger et al, 1995
	protein	Chouinard et al, 2004
UGT2B17	mRNA	Beaulieu et al, 1996
	protein	Barbier et al, 2000; Chouinard et al, 2004
Transporters		
MRP1 (ABCC1)	mRNA	Schummer et al, 1999
	protein	Sullivan et al, 1998; DiPaola et al, 2001
MRP3 (ABCC2)	mRNA	Uchiumi et al, 1998; Lee et al, 1998
MRP4 (ABCC4)	protein	Lee et al, 1998
MRP	mRNA	Nooter et al, 1995
	protein	Sugawara et al, 1997
SLC22A3	mRNA	Verhaagh et al, 1999

The multiplicity of the CYP system and the catalytic versatility of these enzymes give rise to the broad range of substrates that undergo phase I oxidation in tissues. CYPs are a group of very similar enzymes that are subdivided into families (indicated by an Arabic numeral) if they are at least 40% related at the amino acid level, and into subfamilies (indicated by a capital letter) if they are at least 55% related (Nelson et al, 1996). In general, drug- and xenobiotic-metabolizing CYPs are restricted to families 1–4, and CYPs from other families mediate the oxidative biotransformation of physiologically important lipophilic substrates, including vitamins A and D, bile acids, androgens, and other steroids. Phase II enzymes include the N-acetyltransferases (NATs), which conjugate oxidized metabolites of arylamines and endogenous amines; glutathione S-transferases (GSTs), which play a key role in the detoxification of reactive electrophiles, including carcinogens or procarcinogens

(Krishna and Klotz, 1994); and UDP-glucuronosyltransferases and sulfotransferases that catalyze the formation of glucuronides and sulfates of phenolic and acidic metabolites.

Disposition of Drugs and Metabolites

Drug or metabolite conjugates are generally too hydrophilic to diffuse out of the cell, and require transporter proteins (pumps) to facilitate their removal. All human transporters identified to date are members of the multidrug resistance protein (MRP) family of adenosine triphosphate (ATP)-binding cassette (ABC) or solute carrier (SLC) superfamilies. The majority of transporters involved in the disposition of drugs and xenobiotics belong to the ABC (P-glycoprotein [P-gp]) transporter family, but some SLC transporters also participate. The driving force for the efflux of drug conjugates against concentration gradients is ATP hydrolysis (Borst and Elferink, 2002). ABC (P-gp) transporters are encoded by the multidrug resistance-1 (*MDR1*) gene and are transmembrane calcium-dependent efflux proteins of molecular weight of approximately 150–200 kd, and have 2 ATP-binding domains, with the exception of the recently described breast cancer resistance protein BCRP/ABCG2 (Rocchi et al, 2000). The MRP family of ABC transporters consists of at least 9 members. MRP1 and MRP2 exhibit the highest potency and broadest resistance profiles regarding natural product agents; MRP3 confers low levels of resistance to etoposide; and MRP4 and MRP5 confer resistance to nucleoside analogs and transport cyclic nucleotides. In contrast, SLC transporters are smaller proteins (50–100 kd) and function by exchanging or cotransporting ions. Overexpression of ABC transporter proteins, such as ABCB1 (also termed MDR1), in cancer cells is associated with acquired multidrug resistance because of the capacity of transporters to extrude anticancer drugs and decrease their intracellular concentration (Ambudkar et al, 2003). This review focuses on the role of drug-metabolizing enzymes and transporter proteins in the biotransformation and disposition of drugs used in the management of prostate cancer and hyperplasia. The expression of these drug-metabolizing enzymes and drug transporters in the human prostate is reviewed in terms of their likely functional importance. Targeting of biotransformation enzymes and transporters in different therapeutic strategies is also discussed.

Drugs for BPH and Prostate Cancer: Biotransformation and Transport

The commonly prescribed drugs for BPH include the 5α-reductase inhibitors and the α-adrenoceptor antagonists. The 5α-reductase inhibitors (eg, finasteride and dutasteride) decrease the production of 5α-dihydrotestosterone, the potent androgen-receptor agonist that is primarily responsible for prostatic enlargement (Boyle et al, 2004). The α-adrenoceptor antagonists (eg, alfuzosin, tamsulosin, doxazosin, indoramin, prazosin, and terazosin) relax the smooth muscles of the prostate gland.

In prostate cancer, several approaches to androgen deprivation are currently used. The steroidal antiandrogen, cyproterone acetate, inhibits gonadotrophin secretion, which decreases testosterone levels. Estrogens also act by a central mechanism to inhibit the secretion of luteinizing hormone–releasing hormone (LHRH), which, in turn, decreases serum testosterone concentrations. The nonsteroidal antiandrogens, flutamide, bicalutamide, and nilutamide, compete with androgens for binding to nuclear receptors in prostate cells (Dearnaley, 1994).

Cytotoxic agents used in chemotherapy for other cancers are also prescribed for their palliative effects in patients with prostate cancer. However, drug resistance is a major problem, resulting in extremely low remission rates from cytotoxic drug therapy. Anticancer drugs in current use include docetaxel, paclitaxel, vincristine, vinblastine, cyclophosphamide, doxorubicin, mitoxantrone, etoposide, *cis*platin, methotrexate, and 5-fluorouracil. Mitoxantrone plus prednisone and docetaxel plus prednisone are drug combinations approved for treatment of metastatic prostate cancer (Dagher et al, 2004; Denmeade and Isaacs, 2004).

The antiprogestin, mifepristone, and the antiestrogen, tamoxifen, are effective inducers of apoptosis that have the potential to overcome intrinsic resistance of androgen-independent prostate cancer cells (El Etreby et al, 2000). Mifepristone has recently been found to be an effective antiandrogen (Song et al, 2004).

Most studies of the metabolism of drugs used for the management of BPH and prostate cancer have been undertaken in liver fractions because the liver contains high levels of enzyme activity. Such studies have provided definitive information on the enzymes and transporters involved in metabolism and transport of these pharmacologic agents. To date, there are very few reports of drug metabolism or drug transport in prostate fractions or prostate-derived cells.

5α-Reductase Inhibitors

The in vitro oxidation of the 5α-reductase inhibitor finasteride used in BPH was studied in human liver microsomes and microsomes containing recombinant human CYPs. Sequential ω-oxidation of finasteride to its carboxylic acid metabolite proceeded via the ω-hydroxy and ω-carboxaldehyde intermediates and was mediated by CYP3A (Huskey et al, 1995); other CYPs that were tested were inactive. Another 5α-reductase inhibitor, dutasteride, was reported to be metabolized by human CYP3A4 (Andriole and Kirby, 2003) (Table 2).

α-Adrenergic Antagonists

Tamsulosin, a potent α-antagonist used for the treatment of BPH, is metabolized to *o*-ethoxyphenoxyacetate and *O*-deethylated products by CYP3A4, and to *m*-hydroxy and *O*-demethylated products by hepatic CYP2D6 (Kamimura et al, 1998). Indoramin undergoes oxidation at the 6-position of the indole ring by as yet unidentified CYPs, followed by sulfonylation at the resultant 6-hydroxyl group; this metabolite is likely to be a transporter substrate (Pierce, 1990). Prazosin is a potent inhibitor of the human organic cation transporters (hOCT), hOCT1 and hOCT3 (SLC22A1 and SLC22A3, respectively), as well as BCRP (ABCG2) (Ozvegy et al, 2001; Hayer-Zillgen et al, 2002). Doxazosin is transported by MDR1 (ABCB1), a process that is inhibited by digoxin (Takara et al, 2002).

Anticancer Agents

A range of cytotoxic agents have been used for the treatment of prostate cancer, with most undergoing CYP3A-mediated oxidation to inactive metabolites, which is consistent with the important role of these enzymes in the oxidation of most pharmaceutical agents. Thus, vinblastine and other vinca alkaloids, including vinorelbine and vindesine, undergo CYP3A hydroxylation; in some cases, a role for CYP3A has been inferred from inhibition of other CYP3A substrate oxidations by the vinca alkaloids (Zhou et al, 1993; Kajita et al, 2000).

Docetaxel metabolism requires CYP3A, with evidence for involvement of both CYP3A4 and CYP3A5 (Shou et al, 1998; Cresteil et al, 2002). There have been reports that docetaxel metabolism is inhibited by coadministration with erythromycin, ketoconazole, nifedipine, midazolam, and troleandomycin, and that it is induced by the classic CYP3A inducers, dexamethasone and rifampicin (Marre et al, 1996). Thus, docetaxel metabolism is likely to be influenced by a wide range of coadministered drugs. Interestingly, it was reported that docetaxel and paclitaxel also inhibited CYP1B1 in vitro (Rochat et al, 2001). This enzyme may only have a minor role in drug metabolism but participates in carcinogen activation. Thus, it may be speculated that docetaxel and paclitaxel may prevent the conversion of chemical procarcinogens to toxic species, but studies that are more extensive are now required to test this hypothesis.

In contrast with docetaxel metabolism, which seems to be extensively CYP3A-mediated, formation of the principal 6α-hydroxylated metabolite of paclitaxel is catalyzed by CYP2C8 (Cresteil et al, 1994; Rahman et al, 1994). CYP3A4 has a role in paclitaxel metabolism but catalyzes the formation of only the minor *p*-hydroxyphenyl metabolite (Cresteil et al, 1994; Harris et al, 1994; Kumar et al, 1994). The topoisomerase inhibitors etoposide and teniposide are also substrates of CYP3A4; thus, catechol formation and etoposide 3′-hydroxylation have been shown to be highly correlated with midazolam oxidation pathways (Relling et al, 1994; Kawashiro et al, 1998).

Oxidation of the oxazaphosphoramides, cyclophosphamide, ifosfamide, and trofosfamide, involves CYP3A, but the significance of this involvement is quite complex because CYP3A oxidation is a bioactivation process. In the case of cyclophosphamide, 4-hydroxylation produces the active cytotoxic agent, phosphoramide mustard, as well as the ancillary product, acrolein, which possesses significant toxicity (Gurtoo et al, 1981; Sladek et al, 1988; Murray et al, 1994; Huang et al, 2000). A more recent study also implicated *CYP2C* gene products, especially CYP2C9 and CYP2C19, in the activation of oxazaphosphorines (Chang et al, 1997), although their contribution in vivo needs to be confirmed and the potential impact of variant genes should be considered. CYP2B6 has been shown to make a substantial contribution to formation of the neurotoxic metabolite of ifosfamide (Huang et al, 2000).

Tegafur is a prodrug that undergoes CYP2A6-dependent 5′-hydroxylation to generate 5-fluorouracil, the active cytotoxic agent (Daigo et al, 2002). The defective alleles *4C and *11 have less than 50% of the catalytic competence of the wild-type *1 allele. Roles for CYP1A and CYP2C8 have also been suggested (Komatsu et al, 2000), although the relative contributions of the enzymes remain to be determined. However, as an extrahepatic CYP, CYP1A1, in particular, may well contribute to local activation in tissues such as the prostate. In similar fashion to these agents, dacarbazine requires 3-N-demethylation by CYP1As, and possibly also CYP2E1, for its cytotoxic action (Reid et al, 1999; Long and Dolan, 2001). CYP1A2 seems to be the major catalyst, but, again, CYP1A1 may be operative in extrahepatic tissues.

Antiandrogens

CYP1A2 and CYP1B1 catalyze the biotransformation of the antiandrogen flutamide to its 2-hydroxylated metabolite (Shet et al, 1997). Although CYP3A4 makes only a minor contribution to this pathway (Shet et al, 1997), it was implicated in the formation of a reactive metabolite that may be associated with flutamide hepatotoxicity (Berson et al, 1993). It has been speculated that alterations in CYP1A2 function in the prostate may contribute to the decreased flutamide effectiveness that has been noted in some patients.

Other Hormonal Agents

Tamoxifen and toremifene are oxidized by CYPs to pharmacologically active and inactive metabolites and also to reactive products with genotoxic potential (Boocock et al,

Table 2. *Metabolism and transport of pharmacologic agents used in BPH and prostate cancer*

Drug	Enzyme/Transporter Implicated	References
Antiandrogens		
Bicalutamide	Inhibits CYP3A4	Cockshott, 2004
Flutamide	Metabolized by CYP1A and CYP3A	Berson et al, 1993; Shet et al, 1997
	Inhibits CYP1B1	Rochat et al, 2001
5α-reductase inhibitors		
Dutasteride	Metabolized by CYP3A4	Andriole and Kirby, 2003
Finasteridex	Metabolized by CYP3A4	Huskey et al, 1995
	Induces GST-π	Montironi et al, 1999
α-adrenergic antagonists		
Doxazosin	Transported by MDR1	Takara et al, 2002
Indoramin	Metabolized by CYP (unspecified); sulfonation	Pierce, 1990
Prazosin	Inhibitor of hOCT1 and hOCT3	Hayer-Zillgen et el, 2002
	Transported by BCRP	Ozvegy et al, 2001
Tamsulosin	Metabolized by CYP3A4 and CYP2D6	Kamimura et al, 1998
Miscellaneous cytotoxic agents		
Cyclophosphamide	Activation by CYP2Cs	Chang et al, 1997
	Metabolized by CYP3A4	Huang et al, 2000; Murray et al, 1994
Dacarbazine	Activation by CYP1A; CYP2E at higher concentrations	Long and Dolan, 2001; Reid et al, 1999
Docetaxel	Metabolized by CYP3A4 and CYP3A5	Cresteil et al, 2002; Shou et al, 1998
Doxazosin	Transported by MDR1	Takara et al, 2002
Etoposide	Metabolized by CYP3A4	Relling et al, 1994; Kawashiro et al, 1998
	Transported by P-gp	Shapiro and Ling, 1998
Flavopiridol	Transported by ABCG2	Doyle and Ross, 2003
	Metabolized by UGT1A1, UGT1A9, UGT1A10	Hagenauer et al, 2001
Ifosfamide	Activation by CYP2Cs	Chang et al, 1997
	Metabolized by CYP3A4	Huang et al, 2000; Murray et al, 1994
	Metabolized by CYP2B6	Huang et al, 2000
JM216	Inhibited CYP2C8 and CYP3A4	Ando et al, 1998
Liopristone	Metabolized by CYP3A4	Jang and Benet, 1997
Methotrexate	Transported by MRP1-4	Chen et al, 2003
	Transported by ABCG2(BCRP)	Chen et al, 2003; Doyle and Ross, 2003
Mifepristone	Metabolized by CYP3A4	Heikinheimo, 1997; Jang et al, 1996
	Metabolized by CYP3A4 and CYP3A5	Khan et al, 2002
Mitoxantrone	Transported by ABCG2(BCRP)	Cooray et al, 2004; Doyle and Ross, 2003
Navelbine	Inhibits CYP3A4	Zhou et al, 1993
Onapristone	Metabolized by CYP3A4	Jang and Benet, 1997
Paclitaxel	Metabolized by CYP3A4 and CYP2C8	Cresteil et al, 2002
Tamoxifen	α-hydroxylation by CYP3A4	Boocock et al, 2002
	4-hydroxylation by CYP2D6 and CYP2B6 at high substrate concentrations	Boocock et al, 2002; Crewe et al, 2002
	N-demethylation by CYP2D6, CYP1A1, CYP3A4	Crewe et al, 2002
	Trans-cis isomerization by CYP1B1	Crewe et al, 2002

Table 2. Continued.

Drug	Enzyme/Transporter Implicated	References
Tegafur	Prodrug activation (5'-hydroxylation to 5-FU) by CYP2D6	Daigo et al, 2002
	Metabolized by CYP1A2, CYP2A6, and CYP2C8	Komatsu et al, 2000
Teniposide	Metabolized by CYP3A4	Relling et al, 1994
Toremifene	CYP3A4 α-hydroxylation	Kim et al, 2003
Trofosfamide	Metabolized by CYP3A4	May-Manche et al, 1999
Vinblastine	Transported by P-gp	Bruggemann et al, 1992; Garrigues et al, 2002
	Metabolized by CYP3A4	Yao et al, 2000
Vincristine	Inhibits CYP3A4	Zhou et al, 1993
Vindesine	Metabolized by CYP3A4	Zhou et al, 1993
Vinorelbine	Metabolized by CYP3A4	Kajita et al, 2000

2002; Crewe et al, 2002). Several CYPs seem to be involved in these pathways, with the oxidation of tamoxifen as the prototypic agent in this class being particularly well studied; biotransformation of toremifene has been less well investigated (Kim et al, 2003). Studies in nonhuman systems have strongly implicated CYP3A and CYP2C as targets for inhibition by tamoxifen metabolites (Reidy and Murray, 1989); similar inhibitory processes with human CYPs may give rise to clinically relevant drug interactions. There is a major role for CYP3A4 in the α-hydroxylation (genotoxic product) and N-demethylation (inactive product) of tamoxifen; CYP2D6 and CYP1A also contribute to N-demethylation, whereas 4-hydroxylation to the active product primarily involves CYP2D6 and CYP2B6 (Boocock et al, 2002; Crewe et al, 2002). Factors that influence CYP3A, CYP2B6, and CYP1A activity (including induction and inhibition) and CYP2D6 activity (such as genetic polymorphism and enzyme inhibition) greatly affect tamoxifen action.

The antiprogestins, mifepristone, lilopristone, and onapristone, are CYP3A4 substrates (Heikinheimo, 1997; Jang and Benet, 1997; Khan et al, 2002). CYP3A5 also oxidizes mifepristone (Khan et al, 2002) in a process that leads to mechanism-based inactivation of enzyme; interestingly, CYP3A4 was not susceptible to inactivation by this agent. Thus, protracted inhibition of CYP3A5, but not CYP3A4, may occur after mifepristone administration. Khan et al (2002) also showed that CYP3A4 oxidized mifepristone by monodesmethylation and didesmethylation as well as C-oxidation, whereas CYP3A5 only formed the N-desmethyl metabolite.

Transport Processes for Drugs Used in BPH and Prostate Cancer

As well as their requirement for biotransformation before elimination, a large number of anticancer agents are substrates of the P-gp transporter (MDR1/ABCB1), including vinblastine and other vinca alkaloids (Bruggemann, 1992; Zhou and Rahmani, 1992; Garrigues et al, 2002), taxanes (Shirakawa et al, 1999), mifepristone, and possibly also lilopristone, onapristone (Jang and Benet, 1997), and etoposide (Shapiro and Ling, 1998). Some anticancer agents do not require oxidation but are transporter substrates. Methotrexate is transported by MRP1–4 (ABCC1 to ABCC4; Chen et al, 2002) and BCRP (ABCG2; Chen et al, 2003; Doyle and Ross, 2003), whereas mitoxantrone is another substrate of ABCG2 (Doyle and Ross, 2003; Cooray et al, 2004).

Biotransformation Enzymes and Drug Transporters in the Prostate

The detection of several CYPs and transporters in prostate tissue, as well as the responsiveness of some of these genes to coadministered drugs or after exposure to envi-

ronmental chemicals may be highly significant. Local metabolic processes may contribute to keeping the concentration of agents used in the management of prostatic conditions to subtherapeutic levels. This may give rise to emergence of drug-resistant cells and result in the failure of therapy.

Phase I Drug-Metabolizing Enzymes in the Prostate

There is differential expression of drug-metabolizing enzymes in the human prostate (Table 1). Some enzymes (CYP3A4 and CYP3A7) are detected in normal tissue or the benign tissue at the margin surrounding tumors but are absent from neoplastic tissue (Finnström et al, 2001).

CYP1A, CYP2C, and CYP3A, as well as epoxide hydrolase, have been detected in prostate epithelium and in prostate tumor cells (Murray et al, 1995). Finnström et al (2001) described the expression pattern of *CYP* gene transcripts in tissue specimens from BPH and prostate cancer patients. CYP1A2, CYP1B1, CYP2C19, CYP2D6, CYP3A5, and CYP4B1 were detected in normal and tumor samples, but CYP3A4 was only present in normal samples. Of the CYPs measured, CYP1A1, CYP2C9, CYP2E1, and CYP4A11 were not detected by these investigators (Finnström et al, 2001). Similarly, CYP2C but not CYP3A, was detected in normal prostate epithelium and in neoplastic prostate tissue by immunohistochemistry (Yokose et al, 1999). CYP2C9 messenger RNA (mRNA) was detected in the prostate, but mRNA for CYP2C8, CYP2C18, and CYP2C19 were absent (Klose et al, 1999). In contrast to the report of Yokose et al, CYP3A5 has been detected in the prostate (Agundez et al, 1998; Yamakoshi et al, 1999; Finnström et al, 2001). CYP1A, CYP2C, CYP2D, and CYP3A are important catalysts of drug biotransformation. As discussed in the section on metabolism of prostate drugs, these CYPs are involved in their biotransformation. Indeed, Agundez et al (1998) reported that the Michaelis constants (K_ms) for CYP3A substrate oxidation in the prostate were similar to those in liver, notwithstanding the much lower V_{max} values, reflecting the lower level of protein expression.

The polymorphically distributed CYP2D6 has also been detected in the prostate (Agundez et al, 1998). Activity (N-demethylation of dextromethorphan) was approximately 10% of that in liver. It will now be of interest to determine whether the extensive range of allelic variants of CYP2D6 also give rise to the same range of transcripts in the prostate that have been detected in liver.

A novel member of the CYP3A subfamily, CYP3A43, was detected in the adult liver, kidney, pancreas, and prostate by polymerase chain reaction (PCR)/agarose gel electrophoresis. Domanski et al cloned and expressed this enzyme in a heterologous cell system, but its role in drug metabolism has yet to be elucidated (Domanski et al, 2001).

Phase II Drug-Metabolizing Enzymes in the Prostate

GST-α, GST-μ, and GST-π have been detected in non-neoplastic prostate epithelium but only GST-α and GST-μ were detected in prostate tumors (Murray et al, 1995). Conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione as well as Western blot analysis indicated the presence of subunits from each of the major classes of GSTs in normal and BPH prostate tissue (Tew et al, 1987; Di Ilio et al, 1990). Indeed, expression of GST-π has also been reported on a number of occasions (Cookson et al, 1997). GST-π was highly expressed in basal epithelial cells but was present only at a very low level in acinar epithelial cells (Cookson et al, 1997), and was absent in the prostate cancer precursor, prostatic intraepithelial neoplasia (PIN), and in prostate carcinoma, in agreement with the reports of other investigators (Murray et al, 1995; Sullivan et al, 1998). GST-π predominates in basal cells and secretory acinar epithelium of normal and BPH tissues, but is consistently absent in incidental prostate cancer cells, in PIN, and in malignant prostate tumor. GST-π is, however, frequently expressed in other cancers.

Expression of NAT1 and NAT2 isoforms in BPH tissues by in situ hybridization has also been noted (Wang et al, 1999). Transcripts of NAT1 and NAT2 were detected in the glandular prostate epithelium, whereas the loose connective tissue and the capillary vessels of the stroma, which surround the glandular prostate, did not show any significant hybridization signal for these enzymes (Wang et al, 1999).

Drug Transporters in the Human Prostate

MRP1 (ABCC1) expression was high in early stage prostate tumors and increased with more severe (Gleason and surgical) stages/categories of pathology (Sullivan et al, 1998). Several other groups also examined drug transporters in the prostate. Uchiumi et al (1998) reported minimal expression of canalicular multispecific organic anion transporter (cMOAT)-2/MRP3 (ABCC3) as well as of cMOAT1/MRP2 (ABCC2) in the prostate, compared with liver. Lee et al (1998) isolated a cDNA that encodes ABCC4 (also known as MRP4) by a degenerate PCR approach. The MRP4 expression pattern was examined in a range of human tissues by hybridization analysis; of all of the tissues examined, transcript levels were highest in the prostate (Lee et al, 1998). The subcellular localization of ABCC4 was assessed in transfected cells and in prostate tissue by immunohistochemistry; ABCC4 protein was localized primarily in the basolateral plasma membrane of tubuloacinar cells of prostate tissue. Based on this location, it was suggested that one function of ABCC4 might be to efflux xenobiotics/toxins out of pros-

tatic epithelial cells into the stroma to protect the prostatic fluid (Lee et al, 2000). Kawai et al also detected MDR1 (ABCB1) in both benign and malignant prostate cells while clarifying the possible role of the transporter protein P-gp in the intrinsic drug resistance of prostate cancer cells (Kawai et al, 2000). Similar findings were reported by David-Beabes et al (2000). In nonmalignant prostate glands, ABCB1 expression in the epithelial cells was highest in the inner rather than in the outer zone. It was suggested that intrinsic drug resistance may be related to overexpression of MDR1.

Drug Resistance in the Prostate: Role of Biotransformation Enzymes and Transporter Proteins

Drug resistance in tumor cells has been associated with altered expression of transporter proteins and drug-metabolizing enzymes. Thus, overexpression of these genes enables the target cells to keep intracellular drug concentrations low. CYP overexpression may be particularly relevant to the susceptibility of prostate cells to therapeutic regimens. Ly et al (1999) demonstrated that prostate cells were resistant to the antiproliferative effects of 1α, 25-dihydroxyvitamin D3 and tested the hypothesis that this may be because of enhanced activity of CYP24 (a vitamin D3 hydroxylase). Coadministration of liarozole, an inhibitor of CYP-dependent vitamin D and vitamin A hydroxylation, effectively restored the sensitivity of the cells to 1α, 25-dihydroxyvitamin D3 (Ly et al, 1999). In a similar strategy, coadministration of the imidazole, ketoconazole, and of imidazole-based steroidal derivatives decreased androgen production in LNCaP cells by inhibiting the CYP17 hydroxylase, which is a critical enzyme in the production of androgens from C21 precursor steroids (Klus et al, 1996). This is analogous to treatment of cells with antiandrogens such as flutamide, but which targets an earlier step in androgen biosynthesis.

The antimycotic ketoconazole has been evaluated for the management of hormone-refractory prostate cancer; a recent trial yielded favorable results (Wilkinson and Chodak, 2004). Ketoconazole blocks both testicular and adrenal androgen biosynthesis by inhibiting CYP enzymes involved in steroidogenesis (Pont et al, 1982). Like similar strategies to inhibit androgen production, the use of potent CYP inhibitors to target steroidogenic pathways may be viable, although the nonselectivity of ketoconazole as a broad-spectrum CYP inhibitor will most likely preclude its widespread use. These reports establish that nondrug-metabolizing CYPs in prostate cells may have critical roles in homeostasis and offer potentially valuable and novel approaches to the treatment of prostate disorders using selective inhibitors.

The transporter MRP1 (ABCC1) seems to have a role in cellular resistance to the antiandrogen flutamide (Grzywacz et al, 2003). In the MRP-1 overexpressing cell lines (KB4D–10 and PC-3-ADR) intracellular flutamide and hydroxyflutamide concentrations are extremely low. Overexpression of MRP-1 in resected prostate cancer tissue (Sullivan et al, 1998) may shed light on the in vivo significance of the resistance of cultured cells to antiandrogens. Inhibition of MRP-1 (eg, by leukotriene D4, buthionine sulfoximine, and VX-710) may be of value in management of prostate cancers with antiandrogens (Grzywacz et al, 2003).

MRP3 (ABCC3) was investigated in the prostate cell lines PC-3, P/CDP5 (*cis*platin resistant) and P/CDP5-R (*cis*platin-sensitive revertant); there was no specific change in cellular levels with decreasing accumulations of *cis*platin (Uchiumi et al, 1998). The drug transporter MRP4 (ABCC4) conferred resistance to short-term methotrexate treatment (Lee et al, 2000).

Single nucleotide polymorphisms (SNPs) were identified in *MRP1–4* (*ABCC1* to *ABCC4*) genes, among many others (Saito et al, 2002). Various mutations in *MDR1, MRP1,* and *MRP2* were observed (Ito et al, 2001), whereas polymorphisms in *MRP3* displayed interethnic differences in incidence (Lang et al, 2003). To date, more than 28 SNPs have been found in *MDR1* (Sakaeda et al, 2003), although the functional impact of these variants remains largely unclear. However, increasing evidence implicates such genetic polymorphisms in interindividual differences in activities of drug transporters.

Role of Biotransformation in Chemical Carcinogenesis in the Prostate

Because drug-metabolizing enzymes are involved in the activation of carcinogens and mutagens, several studies have evaluated the association between their expression and the expression of gene variants as risk factors for carcinogenesis. Thus, polymorphisms have been proposed as genetic biomarkers for susceptibility to some forms of cancer, for instance, NAT2 polymorphism is proposed as a biomarker for prostate cancer because it has a proven association with more clinically advanced and pathologically aggressive disease (Hamasaki et al, 2003).

Epidemiologic studies have implicated an association between the intake of charbroiled (barbecued) beef meat and the incidence of prostate cancer (Williams et al, 2000; Ferguson, 2002). Meat cooked in this way contains food-derived mutagens that are activated by CYPs to proximate mutagenic species that bind to DNA. Thus, CYPs oxidize 2-amino-3-methylimidazo[4,5-*f*]quinoline and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) to hydroxylated products that are activated further by NAT-conjugating enzymes to reactive DNA-binding intermediates (Turesky et al, 1991). Such adducts may escape DNA repair processes, leading to permanent damage to genetic material. Biotransformation enzymes clearly have a major role in promutagen bioactivation as well as in the meta-

bolic conversion of drugs to inactive and active metabolites.

Ingestion of chargrilled meat also results in induction of CYP1A enzymes (Fontana et al, 1999). CYP1A1 contributes significantly to metabolic activation of heterocyclic and aromatic amines and polyaromatic hydrocarbons. CYP1A2 was postulated to activate carcinogens in BPH tissue, and polymorphisms in the gene have been implicated in prostate cancer (Murata et al, 2001). Metastatic prostate cell lines (LNCaP, DU145, and PC-3) showed constitutive expression of CYP1A1 and CYP1A2. Incubation with benzo[a]pyrene only induced CYP1A1 and CYP1A2 expression in androgen-dependent cell lines, for instance, LNCaP and 1542-CP3TX. Thus, it was suggested that aromatic (aryl) hydrocarbon receptor ligands contribute to the development and progression of prostate cancer by upregulating CYPs that activate hydrocarbons to their ultimate carcinogenic metabolites (Sterling and Cutroneo, 2004).

Metabolism of carcinogens by CYPs has also been linked to initiation of cancer, for instance, in the esophagus (Murray et al, 1994b; Nakajima et al, 1996) and stomach (Murray et al, 1998), where CYP1B1 is overexpressed. The significance of CYP1B1 expression in prostate tumor cells, however, needs further scrutiny. A possible association between prostate cancer and the defective alleles of CYP2D6, which are responsible for the poor metabolizer phenotype has been suggested (Febbo et al, 1998).

Both CYP3A4 and CYP3A5 have been shown to play a minor role in the activation of food-derived heterocyclic amines (Windmill et al, 1997). The CYP3A4-V allele was associated with a higher tumor to lymph node metastasis stage (TNM) and Gleason grade (Rebbeck et al, 1998). CYP3A4*1B was associated with markers of advanced disease (Keshava et al, 2004; Tayeb et al, 2004; Zeigler-Johnson, 2004). CYP3A43 has been associated with familial prostate cancer. Indeed, the variant CYP3A43*3 has been linked to the severity of prostate cancer (Zeigler-Johnson, 2004).

CYP4B1 mediates the activation of arylamines by N-hydroxylation (Windmill et al, 1997). High expression of CYP4B1 increases the risk of bladder tumor (Imaoka et al, 2000), and may be associated with prostate cancer risk. Indeed, there is a high coincidence of prostate cancers in patients with bladder cancer (Kinoshita et al, 2004).

GST-α and GST-π have been shown to inhibit adduction of activated PhIP metabolites to DNA in cell-free systems (Nelson et al, 2001). Loss of GST-π is proposed to be significant in the development and progression of prostate cancer, because a striking decrease in the expression of the gene *GSTP1* may be relevant for prostate carcinogenesis (Lee et al, 1994). Hypermethylation of *GSTP1* CpG islands was reported to be responsible for the loss of expression of this gene in human prostate cancer cells (Lin et al, 2001), an extremely frequent change in prostatic carcinoma (Santourlidis et al, 1999). Thus, a screening test based on GSTP1 methylation is proposed as an adjunct to serum screening tests and digital rectal examination to identify men at increased risk of harboring cancer despite a negative biopsy and to discriminate prostate carcinoma from BPH (Chu et al, 2002; Gonzalgo et al, 2002; Harden et al, 2003). In addition, the genotypes of GSTs, especially the Val-allele of *GSTP1,* were associated with familial prostate cancer risk (Nakazato et al, 2003). GSTM1 and GSTT1 have also been implicated in prostate cancer (Murata et al, 2001).

Conclusions and Future Perspectives

There is sufficient evidence that enzymes active in phase I and phase II metabolism of xenobiotics are expressed in the prostate. The pharmacokinetics of drugs with high affinity to the prostate gland may be significantly affected by the presence of drug-metabolizing enzymes. However, the extent of the contribution of the prostate to drug metabolism in vivo, as well as the clinical relevance of the presence of drug-metabolizing enzymes, have to be explored in further studies. Identification of individuals at high risk of susceptibility to prostate cancer through detection of polymorphic drug-metabolizing enzymes is underway to complement other diagnostic procedures, with a view to prevention and early treatment of prostate cancer.

As with drug-metabolizing enzymes, the protective role of transporter proteins in the prostate is evident, though their organ-specific role has not been fully elucidated. An understanding of the function of transporter genes may eventually explain individual differences in susceptibility to BPH and prostate cancer, as well as in response to pharmacotherapy. Identification of specific transporters that play a role in the disposition of a drug can be a valuable tool in optimizing efficacy. Reversal agents that can modulate drug resistance mediated by transporters are now being developed. The development of molecules that inhibit transporters that are expressed at high levels in the prostate may also optimize drug efficacy.

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