20 ADME of Anticancer Drugs

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20.1 SUMMARY

Cytochrome P450 (P450 or CYP) comprises a superfamily of heme-thiolate monooxygenases involved in the oxidation of a large number of endogenous and exogenous compounds [1]. In human liver, CYP3A4 is the major P450 enzyme, followed by CYP2C9 [2]. An important role for polymorphic CYP3A5 in drug oxidation in African and Asian populations has been suggested [3,4]. CYP2D6 and CYP2C19 also catalyze the oxidation of many marketed drugs [5], but their content in human liver is relatively low [6]. Recently, CYP3A enzymes have been identified as being involved in the biotransformation of many anticancer drugs, as shown in Table 20.1. Large interindividual variations in the contents and activities of several P450 forms in human livers lead to different roles of P450s in the oxidations of substrates associated with pharmacological or toxicological actions [1].

Pharmacogenetics is a rapidly developing field, especially in oncology. In an ideal future scenario, pharmacogenetics will allow oncologists to personalize therapy based on patients' individual germline genetic test results. This would help improve efficacy, reduce toxicity, and predict nonresponders in a way that would allow an alternative approach to be chosen or individual dose adjustments to be made. Multiple pathways of drug metabolism have been studied extensively, for example, variations in the *UGT1A* gene and the *ABCC2* gene influence irinotecan metabolism and disposition [7]. Other genetic changes result in reduced DNA repair capacity related to platinum efficacy

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TABLE 20.1 Drug-Metabolizing Enzymes Involved in Biotransformation of Anticancer Drugs

Drug-Metabolizing Enzymes	Anticancer Drugs
P450 2A6	Anastrozole, fadrozole, letrozole, tegafur
P450 2B6	Cyclophosphamide, ifosfamide
P450 2C8	Paclitaxel
P450 2D6	Tamoxifen
P450 3A4	Cyclophosphamide, docetaxel, etoposide, gefitinib, ifosfamide, imatinib, irinotecan, retinoic acid, tamoxifen, teniposide, vinca alkaloids
UGT1A1	Irinotecan
DPYD	5-Fluorouracil
TPMT	Azathioprine, mercaptopurine

or reduced cytochrome CYP2D6 activity related to tamoxifen efficacy. Despite the extensive number of pharmacogenetic studies and their promising results, it is still unclear when and how pretreatment genetic screening will be implemented in oncology. Future prospective studies should focus on the effects of pharmacogenetics on patient outcome and then combine this with evaluations of cost effectiveness. In this section, recent findings on anticancer drug metabolism are summarized.

20.2 TEGAFUR AND CYP2A6

Tegafur [5-fluoro-1-(2-tetrahydrofuryl)-2,4($1H$, $3H$)-pyrimidinedione], an anticancer prodrug of 5-fluorouracil (5-FU), has been used clinically for over 30 years for chemotherapy [8]. It has been reported that tegafur is bioactivated to 5-FU (Fig. 20.1) mainly in the liver and that 5-FU may inhibit the growth of cancer cells by the inhibition of thymidylate synthase or by its incorporation into RNA [9]. 5-FU is further biotransformed to an inactive molecule by dihydropyrimidine dehydrogenase (DPYD) in the liver cytosol [10]. Two pathways have been proposed for the conversion of tegafur to 5-FU, the major pathway occurring in the microsomal fraction and the minor pathway in the cytosolic fraction [11]. Liver microsomal 5-FU formation from tegafur is catalyzed by the phase I drug-metabolizing enzymes CYP2A6, CYP1A2, and CYP2C8 [12,13]. On the other hand, cytosolic 5-FU formation from

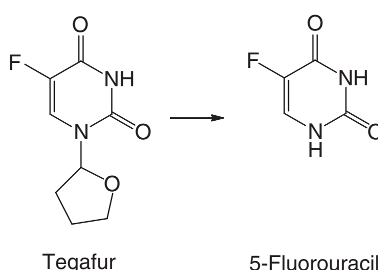

Figure 20.1 Tegafur and its metabolite, 5-fluorouracil.

tegafur is thought to be catalyzed by thymidine phosphorylase [14]. Thymidine phosphorylase, purified from human gastric tumor tissues, has been shown to catalyze 5-FU formation from tegafur as well as thymine formation from thymidine [15]. Thymidine phosphorylase levels were reported to be higher in various tumor tissues than in normal tissues [16]; therefore, 5-FU formation from tegafur in tumor tissues is considered to be mainly catalyzed by thymidine phosphorylase. The involvement of human liver microsomes and cytosol in 5-FU formation from tegafur in the presence of 5-chloro-2,4-dihydroxypyridine, a DPYD inhibitor, has been reported [17]. Moreover, it was confirmed that cytosolic 5-FU formation is mainly catalyzed by thymidine phosphorylase, not by uridine phosphorylase.

Tegafur has been clinically used for the treatment of various cancers. Recently, it has been used in combination with modulators such as 5-chloro-2,4-dihydroxypyrimidine (CDHP) and potassium otastat [18]. Many enzymes are known to be involved in the metabolism of tegafur, although P450 and dihydroxypyrimidine dehydrogenase are rate-limiting enzymes activating tegafur to 5-FU and inactivating 5-FU, respectively [10,19].

If a cancer patient lacks CYP2A6 activity, the patient is expected to have a poor metabolic phenotype in terms of the efficacy of tegafur. In a clinical study, a newly developed anticancer drug, TS-1 capsules, containing tegafur and CDHP, an inhibitor of DPYD, were orally administered to several gastric cancer patients [20]. The total area under the plasma tegafur concentration–time curve (AUC) in one patient was fourfold higher than that for the other patients. From this subject, a novel CYP2A6 mutation was found that causes an amino acid change from serine to proline at residue 224 [20]. It was concluded that the poor metabolic phenotype of this patient was caused by the existence of two mutant alleles: *CYP2A6*4* (whole deletion of *CYP2A6*) and a new variant, *CYP2A6*11* [20].

20.3 LETROZOLE AND CYP2A6

Aromatase inhibitors directed toward CYP19A1 are a good alternative to anti-estrogens in the treatment of hormone-dependent breast cancer. Aromatase (or CYP19A1) is the enzyme responsible for the rate-limiting step in estrogen synthesis and it is by inhibiting CYP19A1, and as a consequence inhibiting estrogen formation, that aromatase inhibitors present pharmacological activity [21]. The aromatase inhibitors used today, that is, anastrozole, letrozole (4,4′-[1*H*-1,2,4-triazol-1-ylmethylene]bis-benzonitrile, Fig. 20.2), and exemestane, are third-generation drugs, all having high specificity for the aromatase enzyme [22].

Letrozole is an orally active, nonsteroidal competitive inhibitor of aromatase [23] or CYP19A1. CYP19A1 catalyzes the three-step oxidation of androgens to estrogens [24], and its expression is known to be relevant to estrogen-dependent tumors [25]. Although letrozole is currently used worldwide for treatment of advanced breast cancer in postmenopausal women [26], knowledge is still limited regarding the roles of different P450 isoforms, including the pharmacological target site (CYP19A1), in drug disposition in the therapeutic setting.

After a single oral dose of 2.5 mg to human subjects, the mean maximum plasma concentration of letrozole was 13 μM [27]. Owing to the slow elimination half-life, plasma concentrations significantly accumulate with repeated doses, achieving mean

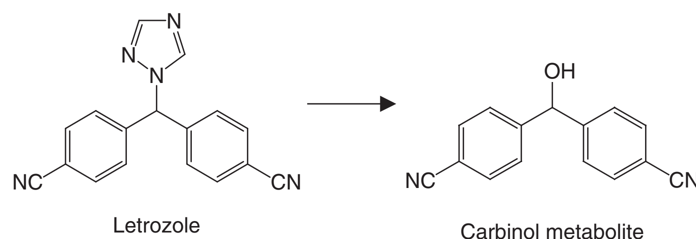

Figure 20.2 Letrozole and its metabolite.

trough and maximum concentrations of 0.3 and 0.5 μ*M*, respectively, at the steady state [23]. In clinical studies, nonlinear pharmacokinetics have been observed for letrozole at high single doses (up to 30 mg) or after repeated doses (up to 10 mg per day) [23], resulting in an ∼10−fold higher plasma exposure than the therapeutic plasma levels in subjects given clinical doses. Large intersubject variation in plasma concentration has generally been observed, regardless of the dose range or regimen used.

Letrozole is cleared from the body by oxidative metabolism to a pharmacologically inactive carbinol metabolite (Fig. 20.2). More than 85% of administered letrozole has been recovered as the glucuronide of the carbinol metabolite [27,28]. Two P450 forms, CYP2A6 and CYP3A4, are reported to mainly contribute to the metabolism, based on *in vitro* biotransformation studies [29,30], but the quantitative contributions of each P450 isoform to the overall clearance, or the contributions of other enzymes such as CYP19A1 (the pharmacological target), remain unclear.

The metabolic activities of individual human liver microsomes showed a significant correlation with coumarin 7-hydroxylase activities (CYP2A6 marker) at a letrozole concentration of 0.5 μ*M*, and a good correlation was also seen with testosterone 6β-hydroxylase activities (CYP3A4 marker) at a substrate concentration of 5 μ*M*. Significantly low carbinol-forming activities were observed in human liver microsomes from individuals possessing *CYP2A6*4/*4* (whole *CYP2A6* gene deletion) at a letrozole concentration of 0.5 μ*M*. The V_{max}/K_m value measured for CYP2A6.7 (amino acid substitution type) in human liver microsomes in the presence of anti-CYP3A4 antibodies was approximately sevenfold smaller than that for CYP2A6.1 (wild type). CYP2A6 and CYP3A4 are recognized as catalyzing the conversion of letrozole to its carbinol metabolite *in vitro*, at low and high concentrations of letrozole, respectively [30]. In addition, CYP2A6 has been recently identified as a polymorphic P450 resulting in poor metabolizers associated with the whole gene deletion (*CYP2A6*4*) and/or activity-depressed genes (*CYP2A6*7* and *CYP2A6*9*), which are known to be more abundant in the Asian population [31] than in Caucasian or African populations [32]. Polymorphic variation of CYP2A6 is considered to be relevant to intersubject variation in the therapeutic exposure of letrozole.

20.4 TAMOXIFEN AND CYP2D6

Tamoxifen (Fig. 20.3) is an estrogen receptor (ER) modulator used worldwide in the prevention and treatment of ER-positive breast cancers. The clinical benefit of this agent for the treatment of ER-positive early breast cancer is evident by the eminent

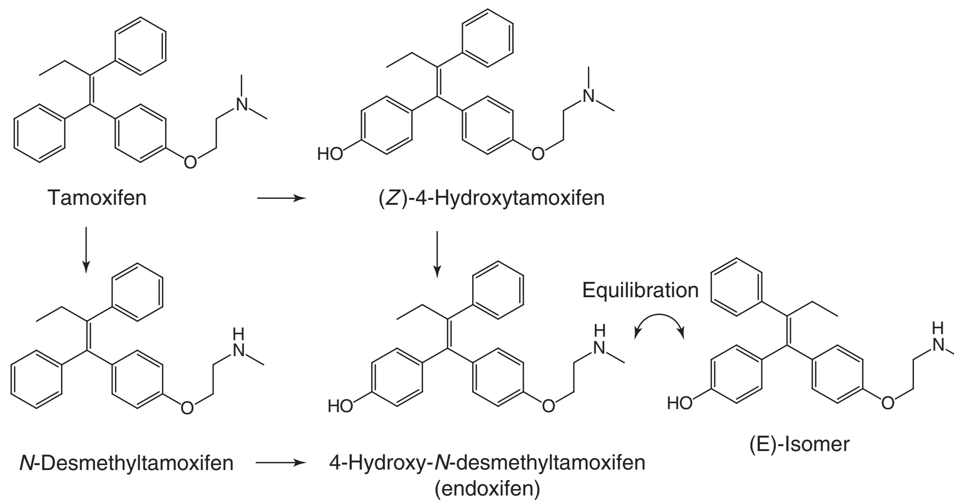

Figure 20.3 Tamoxifen and its metabolites.

reduction in recurrence and mortality rates [33,34]. Despite this success, the mechanisms underlying the ineffectiveness of tamoxifen in a subset of patients are not fully understood; interindividual differences in the formation of active metabolites could be an important factor affecting variability in the response to tamoxifen.

Tamoxifen is a prodrug that requires metabolic activation, carried out mainly by CYP2D6, to elicit its pharmacological activity with clinical outcome [7]. The metabolites 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen (endoxifen) are believed to be the active therapeutic moieties (Fig. 20.3). Compared with the parent drug, these two metabolites have 100-fold greater affinity to ER and 30- to 100-fold greater potency in suppressing estrogen-dependent cell proliferation [35–37].

The *CYP2D6* gene locus is extremely polymorphic, having over 75 allelic variants (http:www.cypalleles.ki.se/cyp2d6.htm), and this contributes to the wide interindividual and ethnic differences in CYP2D6 activity *in vivo*. Women with a poor metabolizer phenotype with respect to CYP2D6, and consequently with low production of the active metabolite, have a greater risk of breast cancer relapse while on tamoxifen, both as a result of genetic variation (e.g., *CYP2D6*4*) and also as a result of enzyme inhibition (e.g., by intake of CYP2D6 inhibitor drugs). In Asians, the major defective allele, *CYP2D6*10*, causes decreased CYP2D6 activity, and a significantly reduced disease-free survival has been shown for *CYP2D6*10* homozygous patients treated with tamoxifen [38–40] in addition to the minor frequency of the whole gene deletion (*CYP2D6*5*). In contrast, an opposite relationship between outcome of tamoxifen treatment and CYP2D6 polymorphism has been published [41]. The basis for this difference is not yet evident. Endocrine therapy tailored to CYP2D6 genotype could be considered for women diagnosed with breast cancer.

Tamoxifen metabolism is mediated by several P450 enzymes, of which CYP2D6 is the most prominent. However, the *in vitro* participation of CYP2C19 in the conversion of tamoxifen to (*Z*)-4-hydroxytamoxifen, *N*-desmethyltamoxifen, and (E)-4-hydroxytamoxifen and in the trans to cis isomerization of 4-hydroxytamoxifen is well documented (Fig. 20.3), especially at high concentrations [42,43]. In addition, patients

carrying the *CYP2C19*17* rapid activity allele [44] have been shown to have a more favorable clinical outcome during tamoxifen therapy, as measured by the relapse-free time [45]. It is noteworthy that two groups have investigated the effects of the defective *CYP2C19*2* allele on tamoxifen treatment [45,46] but found no significant difference as compared to the wild-type allele, indicating that the *CYP2C19*17* allele might be linked to another polymorphism influencing the phenotype/outcome of the treatment. It is interesting to note that an increased number of *CYP2C19*17* alleles correlated with a decreased risk of suffering from breast cancer [47]. These findings collectively suggest that more studies are needed before application of CYP2D6 and CYP2C19 pharmacogenetics to tamoxifen prescription can become a reality. Large prospective studies in this area are necessary to determine the benefit of *CYP2D6* genotyping in the selection of ER modulators for breast cancer.

20.5 THALIDOMIDE AND CYP3A4/5

Thalidomide [α-(*N*-phthalimido)glutarimide] (Fig. 20.4) was withdrawn from clinical use in the early 1960s because of its teratogenic effects in humans but has been approved for the treatment of refractory multiple myeloma by the United States and Japan since 2000 [48,49]. The CYP2C19 genotype is reported to be associated with cancer treatment outcome using thalidomide [50], and the clinical response rate in 62 patients undergoing thalidomide (with dexamethasone) treatment was twice as high in extensive metabolizers than in poor metabolizers [50]. Decreased formation of active thalidomide metabolites (if those contribute to therapy) would be expected with defective CYP2C19 alleles compared to the wild type [50]. No evidence has been obtained for drug interactions between thalidomide and hormonal contraceptives [51,52].

Thalidomide-inhibited CYP2C19-dependent (*S*)-mephenytoin 4′-hydroxylation at high concentrations but enhanced CYP3A5-dependent midazolam hydroxylation and

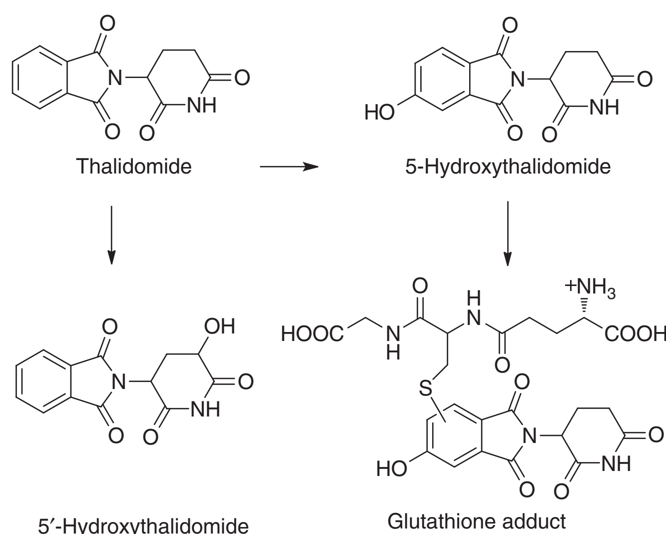

Figure 20.4 Thalidomide and its metabolites.

cyclosporine A clearance at clinically relevant concentrations [53]. Two hydroxylated metabolites of thalidomide (5-hydroxythalidomide and 5′-hydroxythalidomide) are reportedly formed at very low concentrations after incubation with recombinant human CYP2C19 [54]. Recently, although ligand cooperativity with CYP3A5 similar to that for CYP3A4 has been observed [55] and homotropic cooperativity of CYP3A5 with thalidomide was shown [56]. Liquid chromatography–mass spectrometry analysis revealed formation of a glutathione conjugate from (*R*)- and (*S*)-5-hydroxythalidomide, a process catalyzed by liver microsomal CYP3A4 and CYP3A5 in the presence of glutathione (assigned as a conjugate of 5-hydroxythalidomide formed on the phenyl ring) (Fig. 20.4) [56]. These findings established that human CYP3A4 and CYP3A5 mediate thalidomide 5-hydroxylation and further oxidation leads to a glutathione conjugate, which may be of relevance in the pharmacological and toxicological actions of thalidomide.

20.6 IRINOTECAN AND UDP-GLUCURONOSYLTRANSFERASE 1A1

Irinotecan is mainly used in the treatment of colon cancer, particularly in combination with other chemotherapy agents. Irinotecan (known as *CPT-11* during development), a semi-synthetic analogue of the natural alkaloid camptothecin, is activated by enzymatic hydrolysis to SN-38 (Fig. 20.5), an inhibitor of topoisomerase I. The inhibition of topoisomerase I by the active metabolite SN-38 leads to inhibition of both DNA replication and transcription. SN-38 is inactivated by glucuronidation by uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1).

Subjects harboring the *UGT1A1*28* gene (called TA_7 in the promoter region) express fewer UGT1A1 enzymes in their livers and often suffer from Gilbert's syndrome [57]. During chemotherapy, these patients effectively receive a larger than expected dose because their bodies are unable to clear irinotecan as fast as other subjects; this corresponds to higher incidences of severe diarrhea and neutropenia [58]. A clinical study was performed in 2004 that validated prospectively both the association of *UGT1A1*28* with greater toxicity in patients undergoing treatment with irinotecan and the ability of genetic testing to predict toxicity before chemotherapy administration [59]. The US FDA made changes to the labeling of irinotecan to add pharmacogenomics recommendations regarding which patients carrying genetic polymorphism of the *UGT1A1* gene

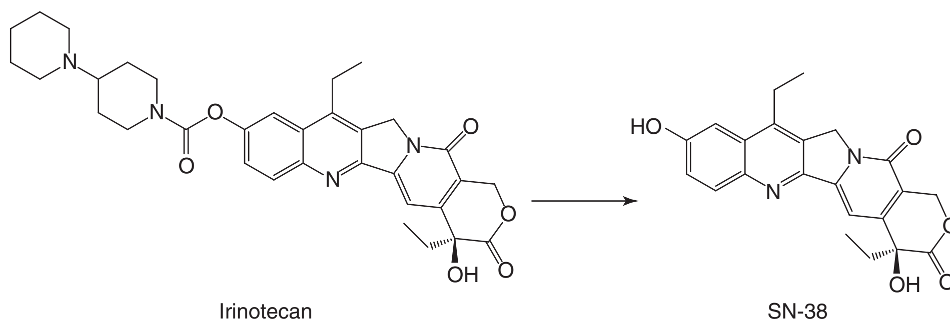

Figure 20.5 Irinotecan and its metabolite.

(specifically *UGT1A1*28*) might benefit from reduced drug doses. Irinotecan is one of the first widely used chemotherapy agents for which personalized medicine according to genotype has been applied [60].

20.7 6-MERCAPTOPURINE AND THIOPURINE-*S*-METHYLTRANSFERASE

6-Mercaptopurine (Fig. 20.6) is used to treat leukemia and pediatric non-Hodgkin's lymphoma and is an immunosuppressive agent [61]. 6-Mercaptopurine inhibits purine nucleotide synthesis and metabolism and alters the synthesis and function of RNA and DNA. Some of the adverse reactions of taking 6-mercaptopurine include black or tarry stools (melena), bloody stools, and bloody urine [62].

6-Mercaptopurine causes myelosuppression, suppressing the production of white and red blood cells and may be toxic to bone marrow. Weekly blood counts are recommended for patients on 6-mercaptopurine, and treatment is interrupted if there is an unexplained abnormally large drop in white blood cell or any other blood count. Subjects who exhibit myelosuppression or bone marrow toxicity should be tested for thiopurine-*S*-methyltransferase (TPMT) enzyme deficiency [63,64]. Although patients with TPMT deficiency are much more likely to develop dangerous myelosuppression [65], it may be possible to continue using 6-mercaptopurine at a lower dose [66].

20.8 CARBOPLATIN AND HYPERBARIC OXYGENATION

Carboplatin (Fig. 20.7), *cis*-diammine-1,1-cyclobutane dicarboxylate platinum(II), is a second-generation platinum antineoplastic agent with modest activity against brain tumors [67]. The efficacy of intravenous administration of 400-mg carboplatin/m^2

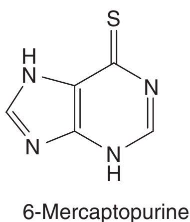

Figure 20.6 Chemical structure of 6-mercaptopurine.

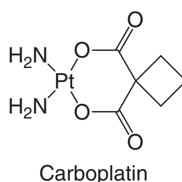

Figure 20.7 Chemical structure of carboplatin.

body surface area over 60 min combined with hyperbaric oxygenation (HBO) therapy (0.2 MPa for 60 min) was investigated in several patients with malignant or brainstem gliomas [68]. Plasma ultrafiltrate samples were analyzed by high performance liquid chromatography [69] to evaluate the relationship between efficacy and pharmacokinetics. Brain tumor response was evaluated by magnetic resonance imaging as a function of maximum plasma concentration, area under the curve, or mean residence time (MRT) for carboplatin. The MRT for carboplatin in the complete or partial response group was significantly longer than that in the progressive disease group [68], but maximum plasma concentration and area under the curve had no effect on outcome. These results suggest that HBO therapy prolongs the biological residence time of carboplatin with modification of chemotherapy and/or clinical antitumor effects in patients with malignant gliomas.

Carboplatin had low brain penetration potential in a cultured blood–brain barrier system. Therefore, HBO therapy at 0.2 MPa for 60 min resulted in apparently higher concentrations of carboplatin in the brain than expected. The present findings of good clinical response may suggest that HBO therapy modifies the pharmacokinetics of carboplatin. With a rat model, changes in the pharmacokinetics of carboplatin with HBO therapy were also suggested by the detection of carboplatin in the brain at 60 min (from the start of administration) after HBO treatment at 0.2 MPa for 20 min [70,71]. Oxygen-induced cerebral vasoconstriction could result in some modification of drug pharmacokinetics, presumably because of various biochemical changes in the brain such as inactivation of intracellular enzyme systems or changes in blood flow rates. Any resultant prolonged biological half-life of carboplatin might explain the effect of combined cisplatin/HBO therapy for malignant gliomas.

Carboplatin had similar endothelial permeability to that of doxorubicin or verapamil, typical P-glycoprotein substrates, in an *in vitro* blood–brain barrier system [71]. Increased permeability of P-glycoprotein-dependent carboplatin as a result of HBO (similar to that resulting from verapamil) may rapidly reach pharmacologically significant concentrations in the central nervous system. Moreover, increased distribution of carboplatin to rat brains with the aid of HBO *in vivo* has been reported [70]. Prolonged biological residence time of carboplatin may be achieved by combination with HBO therapy, resulting in improved efficacy against malignant gliomas. The MRT may be important for predicting continuation or modification of chemotherapy and/or clinical antitumor effects on malignant gliomas.

20.9 CONCLUSIONS

P450 enzymes responsible for the activation or inactivation of anticancer drugs are polymorphic, and much research has been directed at elucidating the ways in which genetic polymorphism in the corresponding genes can affect the efficacy of cancer therapy. With respect to anticancer therapy, the influence of CYP2D6 polymorphism on tamoxifen treatment merits additional study to establish the relationship. Further interesting results can be anticipated leading to more detailed labeling of irinotecan with pharmacogenomic recommendations on which patients carrying genetic polymorphism of the *UGT1A1* gene (specifically *UGT1A1*28*) should perhaps receive reduced drug doses. In the future, increased understanding regarding the role of P450 expression will also provide additional information on the ADME of anticancer drugs.

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