Minireview

Pharmacogenetics in Cancer Etiology and Chemotherapy

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Introduction

Drugs and other xenobiotics have effects that may vary greatly among individuals. Such variability in effect may be due to variability in either pharmacokinetics or pharmacodynamics. The major determinant of variability in pharmacokinetics is drug metabolism, which in many cases is due to polymorphisms in the genes for drug-metabolizing enzymes (1). This often results in subpopulations of poor metabolizers of a particular drug due to a genetically determined deficiency of the drug-metabolizing enzyme. The majority of individuals, by contrast, may be classified as normal or extensive metabolizers. Pharmacodynamic polymorphisms are much less common but may be increasingly identified as important determinants of drug effect (2).

Pharmacogenetics and Cancer Etiology

Ever since the discovery of the debrisoquine polymorphism (3), the interest in pharmacogenetics has centered on whether the ability to metabolize xenobiotics has a positive or negative influence on susceptibility to cancer (4). Recently, this interest has expanded to include the etiology of other diseases including rheumatoid arthritis, Parkinson’s disease, and schizophrenia. This area has been reviewed many times, and the literature is characterized by contradictory reports that a polymorphism in a particular enzyme is or is not associated with an altered susceptibility to cancer (5). Thus, there have been reports that polymorphisms in CYP1A1 (6), CYP2E1 (7), CYP2D6 (8), GSTm1 (9), and NAT2 (10) are associated with an altered risk of developing cancers of the lung, bladder, liver, and colon. Conversely, genotypic and phenotypic differences in these enzymes have been reported, in other studies, to have no influence on susceptibility to cancer (11, 12). Large cohort studies of genotypic and phenotypic differences in disease and control groups are plagued by problems of study design and methodology (13). If polymorphism in drug-metabolizing enzymes does have a role in the mechanism of carcinogenesis, it is unlikely to be the only factor, genetic or otherwise (14, 15). Given differences in expression of different enzymes among normal tissues and between normal and tumor cells of the same tissue, it is debatable whether systemic variation in enzyme expression is representative of the xenobiotic metabolizing environment of the tumor.

Pharmacogenetics and Pharmacology of Antineoplastic Agents

TPMT

6-MP is a major component of maintenance therapy for childhood acute lymphoblastic leukaemia. The metabolism of 6-MP to inactive methylated metabolites is mediated by TPMT (16), and a polymorphism in this enzyme has been described and characterized (17), with the gene now cloned and sequenced (18). Recent investigations have identified several inactivating mutations (19), and ethnic (20) and age-related (21) differences in TPMT activity have been described. The impact of genetic variation in activity of TPMT is seen both as intolerance to 6-MP (and azathioprine) in patients who are homozygous for inactivating mutations (22, 23) and as resistance to treatment in those patients with a high activity of TPMT (17, 24). The latter phenomenon is of greater clinical relevance owing to the rarity of absolute TPMT deficiency (less than 1 in 300; Ref. 17). Treatment may be optimized by tailoring the dose to the activity of TPMT or to concentrations of the active 6-thioguanine nucleotide anabolites (both determined in erythrocytes) or by using 6-thioguanine, an analogue that is not a substrate for TPMT. The determination of phenotypic activity of TPMT is confounded by intrasubject variation (16), especially during therapy, and by variation in other enzymes involved in 6-MP catabolism and anabolism (25).

DPD

DPD catalyzes the initial rate-limiting step in the catabolism of 5-fluorouracil to dihydrouracil, which undergoes further reaction to 5-fluorouridopropionate and 2-fluoro-β-alanine (26). Patients who lack this enzyme will suffer life-threatening or fatal toxicity from modest doses of 5-fluorouracil (27–29). Such patients have a 90% reduction in 5-fluorouracil clearance. DPD deficiency is inherited as an autosomal recessive trait, and family members of deficient probands may demonstrate a partial deficiency. It is not known whether such presumed heterozygotes are at increased risk of 5-fluorouracil toxicity.

The gene for DPD has recently been localized to chromosome region 1p22, and at least one mutation has been identified (30, 31). Thus, population screening using genetic approaches may soon be feasible.

However, the clinical importance of DPD deficiency may be reduced by the introduction of potent inhibitors of DPD (32–34). Such inhibitors will essentially make all patients DPD deficient, potentially resulting in effective treatment with small oral doses of 5-fluorouracil (35).

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The abbreviations used are: TPMT, thiopurine methyltransferase; 6-MP, 6-mercaptopurine; DPD, dihydropyrimidine dehydrogenase; ALDH, aldehyde dehydrogenase; GST, glutathione S-transferase; UDPGT, UDP-glucuronosyltransferase; MGMT, O6-methylguanine methyltransferase; BG, O6-benzylguanine.

Cytochrome P-450 Enzymes

CYP2D6. Originally identified by a deficiency in the metabolism of the hypotensive agent debrisoquine (3), the gene and a number of inactivating mutations have been described recently (36). The overall frequency of deficiency in this enzyme is 5–10% (37).

Although this enzyme is the most studied, polymorphically expressed enzyme and a number of substrates have been identified (37); to date, no metabolic pathway of clinical significance for an antineoplastic agent has been reported to be dependent on CYP2D6 activity (38).

CYP2C19. This enzyme is responsible for the polymorphism in S-mephenytoin 4-hydroxylation, observed more than 10 years ago (39). The incidence of deficiency varies from 5–20%, depending on ethnic origin (40). The exact identity of the enzyme has proved elusive, and the gene responsible has been determined only recently (41). CYP2C19 is also implicated in the metabolism of proton-pump inhibitors such as omeprazole (42) and that of nonsteroidal anti-inflammatory drugs (40). Again, despite intensive study, no role for CYP2C19 in the metabolism of anticancer drugs has been reported (38).

CYP2C9. There have been reports of a polymorphism in this enzyme (43). CYP2C9 is involved in the metabolism of the MDR modulator verapamil (44). The clinical significance of this, if any, is yet to be determined, and its impact on chemotherapeutic agents is unknown.

CYP3A4, CYP3A5, and CYP3A7. This subfamily of enzymes, which includes CYP3A3 at present indistinguishable from CYP3A4, is responsible for a large proportion of the therapeutically important pathways of metabolism for many drugs. Substrates show a wide range of structural variation, with a similarly wide repertoire of oxidative reactions performed. Often more than one product may be produced from the same substrate, and two active sites have been identified on CYP3A4 (45). Chemotherapeutic agents that are substrates for this enzyme include ifosfamide (activation and inactivation; Refs. 46, 47), cyclophosphamide (activation; Ref. 46), etoposide (probably inactivation) (48), paclitaxel (inactivation; Ref. 49), and Vinca alkaloids (unknown; Ref. 50), as well as the resistance modifying agents cyclosporin (51) and dex-verapamil (52). A number of studies have reported that both ifosfamide (53, 54) and cyclophosphamide (55) induce their own metabolism. No polymorphism in this phenomenon was apparent, and its clinical significance is unknown.

Although a polymorphism in the activity of CYP3A4, based on the oxidation of nifedipine, has been reported (56), this has not been observed reproducibly (57). Intriguingly, tissue-specific polymorphic expression has been reported for this subfamily of enzymes. CYP3A5 is expressed in only 20–40% of human adult livers (58) but is uniformly expressed in kidney (59). Conversely, CYP3A4 is expressed in only 14% of adult human kidneys (59), despite consistent expression in liver (60). Recently, CYP3A7, once thought to be confined to expression in fetal tissue, has been found to be expressed in a percentage of adult livers (58, 61). The importance of such tissue-specific expression is not yet known, but a role for CYP3A5 in the activation of ifosfamide has been demonstrated (62), and kidney metabolism of this drug may play a role in its nephrotoxicity (63). Expression of CYP3A mRNA in breast (64, 65) and lung (66) tumor cells has also been reported, although expression of protein is not always detectable by Western blotting (67).

ALDH

The ALDH group of enzymes includes mitochondrial forms that are involved in the catabolism of ethanol. The cytosolic ALDH isozymes have been associated with the inactivation of the activated intermediates formed by the metabolism of ifosfamide and cyclophosphamide, resulting in the formation of carboxy metabolites (68). Increased expression of ALDH1 (68, 69) or, more recently, ALDH3 (70) confers resistance to these oxazaphosphorines in tumor cell lines. An apparent polymorphism in the excretion of carboxycyclophosphamide (71, 72) has not been substantiated in subsequent studies with cyclophosphamide (73) or ifosfamide (74). The role of this family of enzymes in the inactivation of oxazaphosphorines is still under investigation, and genetic variation in ALDH1 has been described in an Asian population (75).

GST

This group of enzymes has been implicated to have a number of roles in modulating the activity of antineoplastic agents (76). The major role proposed is that of inactivation of alkylating species by conjugation with glutathione (GS; Ref. 77). Recently, an alternative mechanism, involving binding of drugs and/or their removal from the cell, has been suggested (78). Of the increasingly complex family of types and subtypes, GSTμ1 has been reported to be polymorphically expressed, with 50% of the population showing no expression (79). As well as a role in the etiology of cancer, expression of GSTμ1 has also been suggested to influence response to chemotherapy (80). The exact mechanism has not yet been determined, and these immunohistochemical studies await confirmation by genotyping (81). A polymorphism of GSTθ1 has also been reported, and deficiency of this isoenzyme may lead to an increased susceptibility to DNA damage (82).

N-Acetyltransferases

There are two major enzymes that N-acetyl xanobiotics, NAT1 and NAT2 (83). The latter was one of the first drug-metabolizing enzymes to be demonstrated to be polymorphic, based on early studies with isoniazid (84). The incidence of the slow acetylator phenotype is approximately 60% in the Western population, although only 10% of Asian subjects are deficient. NAT1 has been considered monomorphic, although recent studies suggest that it too may be polymorphic (85).

Although NAT2 polymorphisms have been of long-standing importance to investigators in the carcinogenesis area (84), they have not been of therapeutic concern to oncologists until the development of amonafide, a topoisomerase II inhibitor. The early clinical development of amonafide was notable for a significant divergence in the recommended Phase II dose between trials at Ohio State (250 mg/m²) and M. D. Anderson (400 mg/m²; Refs. 86 and 87). Almost all Phase II trials used a dose of 300 mg/m² daily for 5 days. In conjunction with CALGB trials in breast cancer, Ratain et al. (88) demonstrated that the major determinant of toxicity was the extent of N-
acetylation to an active metabolite, N-acetyl-amonafide. Fast acetylators had much greater toxicity (myelosuppression) than slow acetylators, suggested to be due to the inhibition of N-acetyl-amonafide of aminofad oxidation by CYP1A2. Subsequent studies at the University of Chicago demonstrated that phenotyping of NAT2 with caffeine allowed more precise dosing (89, 90).

**UDPGTs**

Like the cytochrome P-450 system, the UDPGTs consist of multiple enzymes with a common function, conjugation to form a glucuronide. There are thought to be two families, UGT*1 and UGT*2 (91). Patients with Crigler-Najjar syndrome lack UGT1.1, the enzyme primarily responsible for bilirubin conjugation. Because the various isozymes of UGT*1 are formed by alternate splicing of the gene, such patients may lack either all UGT*1 isozymes (if the mutation is in the common 3' area) or just UGT1.1 (92, 93).

A more common abnormality of glucuronidation is Gilbert’s syndrome, present in approximately 5% of the population. Such individuals have elevated serum bilirubin (unconjugated), which is due to a mutation in the promoter for UGT1.1 (bilirubin UDPGT; Refs. 93 and 94).

Although glucuronidation of anticancer drugs occurs commonly, variability in such metabolism has been associated with variability in toxicity (95, 96). Such variability may be especially important in regard to irinotecan, a new topoisomerase I inhibitor approved in the United States, France, and Japan. The major metabolite of irinotecan in humans is SN-38 glucuronide, a secondary metabolite (97). Because the primary metabolite, SN-38, is active, variability in glucuronidation has been suggested to be the major determinant of the dose-limiting toxicity of irinotecan, diarrhea (97, 98). This appears to be due to enhanced biliary excretion of SN-38 in those patients with decreased glucuronidation. Recent studies have demonstrated that the UDPGT isozyme responsible for the metabolism of SN-38 is UGT1.1 (99). Studies are ongoing to determine the clinical significance of this finding, as patients with Gilbert’s syndrome would be predicted to be at increased risk of toxicity.

**MGMT**

This enzyme, also known as O*-alkylguanine alkyltransferase, repairs the lesion in DNA caused by alkylation of guanine by agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea (100). Inactivators of this enzyme, such as BG, have been developed and shown to potentiate the action of alkylating agents (101). However, mutant forms of MGMT, resistant to the action of BG, have been found in tumor cell lines and, more importantly, a polymorphism has been identified in MGMT in approximately 15% of the population (102). The underlying mutation at codon 160 does not affect the activity of MGMT, but the protein product of the mutated gene with arginine at this position is resistant to inactivation by BG (103).

**NAD(P)H:Quinone Oxidoreductase (NQO1, or DT-diaphorase)**

A number of quinone chemotherapeutic agents are known to be activated by NQO1 (104). A polymorphism in this enzyme has been reported; the mutant protein is virtually inactive and undetectable in the cytosol of cells expressing the mutant gene (105). The incidence of this polymorphism in humans was estimated to be 7% homozygous and 43% heterozygous for the mutation (105). The implications for chemotherapy with mitomycin C and other agents have yet to be evaluated.

**Conclusions: Implications for Cancer Chemotherapy**

Given the severe toxicity and low therapeutic index of most cytotoxic agents, it is desirable to precisely dose cancer chemotherapy. Such “precision” has been historically limited to dosing based on height and weight (body surface area), a very imprecise approach (106). Studies evaluating approaches akin to therapeutic drug monitoring have been conducted but have not been able to demonstrate enough of an advantage for full implementation.

In contrast, dosing based on pretreatment phenotyping or genotyping is likely to be useful in selected circumstances, as exemplified by the studies of aminofad (89, 90) and 6-MP (16). Given the low prevalence of poor metabolizers, the clinical impact is only likely to be significant in a minority of patients, but in those few it could prove critical. As new polymorphisms are identified, it is likely that anticancer drugs will be among their substrates. Clinical investigators should, therefore, be cognizant of the potential for such polymorphisms, and drugs with unpredictable toxicity or extensive interpatient pharmacokinetic variability should be studied in depth.

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