PHARMACOGENETICS OF ANTICANCER AGENTS: LESSONS FROM AMONAFIDE AND IRINOTECAN

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ABSTRACT:
Amonafide and irinotecan are anticancer drugs representative of the clinical relevance of N-acetyltransferase (NAT) and uridine diphosphate glucuronosyltransferase (UGT) polymorphisms in cancer chemotherapy, respectively. Amonafide, a substrate for the polymorphic NAT2, has an active metabolite, N-acetyl-amonafide. Using caffeine as a probe, slow and rapid acetylators of amonafide were identified. Fast acetylators experienced greater myelosuppression than did slow acetylators, and a reduced dose of amonafide for fast acetylators has been recommended. A pharmacodynamic model based on acetylator phenotype, pretreatment white blood cell count, and gender has been proposed for dose individualization. The strategy adopted for amonafide is a model for future investigations in pharmacogenetics, although amonafide is no longer in clinical development. SN-38, the active metabolite of irinotecan, is glucuronidated to the inactive SN-38 glucuronide by UGT1A1, the isof orm catalyzing bilirubin glucuronidation. Genetic defects in UGT1A1 determine Crigler-Najjar and Gilbert’s syndromes characterized by unconjugated hyperbilirubinemia. Gilbert’s syndrome often remains undiagnosed and occurs in up to 19% of individuals. Gilbert’s syndrome is due to a homozygous TA insertion in the TATAA promoter of UGT1A1, leading to the mutated (TA) allele. Irinotecan toxicity depends on the individual glucuronidation rate of SN-38. Decreased SN-38 glucuronidating activity has been found in livers obtained from individuals carrying the (TA) allele. A phenotyping procedure for UGT1A1 has not been identified and genotyping of the UGT1A1 promoter in patients receiving irinotecan may identify patients at increased risk of toxicity. A clinical trial at the University of Chicago is ongoing to demonstrate the predictive significance of UGT1A1 genotyping for irinotecan pharmacodynamics.

Genetic polymorphisms in drug metabolism are one of the most important reasons for interpatient variability in responses to drug therapy (Ingelman-Sundberg et al., 1999). This source of variability is becoming better understood and more predictable through the use of different probe drugs (phenotyping) and genetic analyses (genotyping). Since the discovery of debrisoquine hydroxylation deficiency in the 1970s (Dengler and Eichelbaum, 1977), the pharmacogenetic properties of several drug-metabolizing enzymes have been characterized.

Polymorphic metabolism is of clinical relevance when 1) the metabolic pathway is the main factor for drug clearance and drug concentration, 2) the drug has a narrow therapeutic index, and 3) the plasma pharmacokinetics of the drug and its metabolites correlate with activity and/or toxicity. The elucidation of a pharmacogenetic basis for variation in clinical toxicity is increasingly becoming important in cancer chemotherapy (Iyer and Ratain, 1998). Generally, cytotoxic agents are administered at the maximally tolerated doses, with an expectation that up to one-third of patients will have unacceptable toxicity. Thus, a metabolic polymorphism that affects drug clearance often leads to recognizable events, such as life-threatening toxicity. For these reasons, pharmacogenetic screening before cancer therapy may enable the identification of patients at increased risk of toxicity.

This review will focus on the relevance of pharmacogenetics in the use of two chemotherapeutic agents: amonafide and irinotecan. The disposition of these drugs involves conjugation by phase II metabolizing enzymes, N-acetyltransferases (NAT) (amonafide) and uridine diphosphate glucuronosyltransferases (UGTs) (irinotecan), which have been shown to be polymorphic.

Polymorphism in NAT2 Enzyme: The Experience with Amonafide

Two independently regulated and kinetically distinct human NAT enzymes are known to exist, namely NAT1 and NAT2. The NAT enzymes catalyze the acetylation of a wide variety of amines, among which are both arylamine and heterocyclic aromatic amines (Weber, 1997). Substrates of NAT2 include amonafide, hydralazine, isoniazid, procainamide, and sulfonamides (Grant et al., 1991). Interindividual variations in NAT2 function are associated with the classical isoniazid polymorphism (Price Evans et al., 1960). In the human NAT2 gene, nine point mutations have been identified, either occurring alone or in combination to yield an increasing number of mutant alleles (Taningher et al., 1999). However, those accounting for the majority of the slow acetylator phenotypes are relatively few. Decreased function

1 Abbreviations used are: NAT, N-acetyltransferase; APC, 7-ethyl-10-[4-(5-aminopentanoic acid)-1-piperidino]carbonyloxy camptothecin; CN, Crigler-Najjar syndrome; NPC, 7-ethyl-10-[4-amino-1-piperidino]carbonyloxy camptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, SN-38 glucuronide; UGT, uridine diphosphate glucuronosyltransferase.
alleles of NAT2 cause the recessively inherited slow acetylator phenotype (Ohsako and Deguchi, 1990; Blum et al., 1991; Deguchi, 1992; Hickman et al., 1992; Bell et al., 1993). Two mutant NAT2 alleles are required for the slow acetylator phenotype. Rapid acetylators include both homozygous and heterozygous carriers of the wild-type allele, the former having significantly higher acetylation rates (Cascorbi et al., 1995). The variable N-acetylation of the anticancer drug amonafide has directed the attention of oncologists to the pharmacogenetics of N-acetylation in cancer chemotherapy.

NAT2 enzyme polymorphism plays several roles in the metabolism of amonafide. Amonafide (SNC 308847; 5-amino-2-[2-(dimethylamino)ethyl]-1H-benz[de]isoquinoline-1,3(2H)-dione) is a DNA intercalating agent and topoisomerase II inhibitor (De Isabella et al., 1995). Amonafide is extensively metabolized, including N-acetylation by NAT2 to N-acetyl-amonafide (Fig. 1), a metabolite approximately equipotent in vitro with the parent drug (Felder et al., 1987). Based on preclinical studies in tumor model systems, amonafide was chosen by the National Cancer Institute to undergo phase I clinical trials. A phase I trial of amonafide showed that its pharmacokinetic parameters were highly variable but ascribed this to impaired renal function (O’Brien et al., 1991).

Ratain et al. (1991) investigated a phenotyping procedure for acetylation using caffeine as a probe in cancer patients treated with amonafide. Slow and fast acetylators of both caffeine and amonafide were identified. The production of the acetylated metabolite was one of the major determinants of myelosuppression, since fast acetylators had significantly greater toxicity than slow acetylators (Ratain et al., 1991). However, the area under the plasma concentration-time curve of amonafide was significantly greater in fast acetylators, who would be expected to have a higher clearance and a lower area under the plasma concentration-time curve. This appeared to be an unusual finding compared with other drugs metabolized by N-acetylation. For most drugs, slow acetylators are at greater risk of adverse reactions. The unexpected behavior of amonafide was due to the inhibition of amonafide oxidation by N-acetyl-amonafide, since in vitro studies demonstrated that amonafide is a substrate for CYP1A2 (Fig. 1) and amonafide’s oxidation is inhibited by its acetylated metabolite (Ratain et al., 1995).

A phase I study of amonafide dosing based on acetylator phenotype recommended doses of 250 and 375 mg/m$^2$ for fast and slow acetylators, respectively (Ratain et al., 1993). A fixed amonafide dose of 300 mg/m$^2$ was considered inappropriate for all patients, as fast phenotypes would be expected to experience grade 4 toxicity and slow phenotypes may be significantly underdosed (Ratain et al., 1991). Since there was still significant interpatient variability in toxicity at these dose levels, a subsequent study attempted to develop pharmacodynamic models to individualize amonafide’s dosing. The optimal model was defined by acetylator phenotype, pretreatment white blood cell count, and gender (Ratain et al., 1996).

Amonafide is no longer in clinical development for several reasons. It demonstrated activity in breast cancer and leukemia, but its clinical development has been hampered by highly variable and unpredictable toxicity caused, at least in part, by interindividual differences in N-acetylation. For these reasons, the experience with amonafide will probably remain a model for illustrating the potential importance of pharmacogenetic determinants on pharmacokinetics and pharmacodynamics. It may serve as an example for other investigators interested in population pharmacogenetic studies.

Polymorphism in UGT1A1 Enzyme: The Experience with Irinotecan

UGTs are microsomal enzymes catalyzing the glucuronidation of numerous endogenous and exogenous substrates (Burchell et al., 1995). Glucuronidation increases the polarity of the substrates to allow them to be better eliminated from the body. The human UGTs are classified into UGT1 and UGT2 families (Mackenzie et al., 1997). The UGT1 gene consists of at least 13 unique isoforms with variable exon 1 and common exons 2 to 5. Each of the exons 1 is preceded by its own promoter and differentially spliced to the common exons to produce a unique mature mRNA. The UGT1 family is further classified into multiple isoforms, i.e., UGT1A1, UGT1A3, UGT1A4, up to UGT1A12. The UGT1A1 isoform is responsible for the glucuronidation of bilirubin. The clinically relevant polymorphisms related to genetic abnormalities in UGT1A1 are those associated with familial hyperbilirubinemic syndromes such as Crigler-Najjar syndrome type I (CN-I) and type II (CN-II), and Gilbert’s syndrome. CN-I syndrome is rare and is associated with severe unconjugated hyperbilirubinemia (Jansen et al., 1995). Patients with CN syndromes have absent (CN-I) or reduced (CN-II) UGT1A1 activity with corresponding unconjugated serum bilirubin levels of 15 to >50 mg/dl and 10 to 20 mg/dl, respectively (Seppen et al., 1994; Sampietro and Iolascon, 1999). Gilbert’s syndrome is a mild chronic unconjugated hyperbilirubinemia, with serum bilirubin levels usually <3 mg/dl, although higher, lower, and even normal values are not uncommon (Sampietro and Iolascon, 1999). A wide variation in the incidences of Gilbert’s syndrome has been reported, ranging from 0.5 to 19% in various groups (Owens and Evans, 1975; Monaghan et al., 1996, 1997). Gilbert’s syndrome is usually associated with homozygosity for the sequence (TA)$_6$-TAA instead of (TA)$_6$TAA in the promoter region of the UGT1A1 gene, resulting in reduced UGT1A1 expression levels and activity (Bosma et al., 1995; Monaghan et al., 1996).

In addition to (TA)$_6$ and (TA)$_7$ alleles, two new alleles with five and eight TA repeats, i.e., (TA)$_8$ and (TA)$_9$, have been found (Beutler et al., 1998; DiRienzo et al., 1998). These alleles were present in population samples from African ancestry, where they occur at lower frequencies than the (TA)$_6$ allele, with serum bilirubin levels usually <3 mg/dl, although higher, lower, and even normal values are not uncommon (Sampietro and Iolascon, 1999). A wide variation in the incidences of Gilbert’s syndrome has been reported, ranging from 0.5 to 19% in various groups (Owens and Evans, 1975; Monaghan et al., 1996, 1997). Gilbert’s syndrome is usually associated with homozygosity for the sequence (TA)$_6$-TAA instead of (TA)$_6$TAA in the promoter region of the UGT1A1 gene, resulting in reduced UGT1A1 expression levels and activity (Bosma et al., 1995; Monaghan et al., 1996).

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**Fig. 1.** Metabolism of amonafide to N-acetyl-amonafide and N’-oxide-amonafide.
The frequency of allele (TA)7 differs significantly between sub-Saharan Africans and Caucasians (Hall et al., 1999).

There have been recent reports of heterozygous and homozygous missense mutations in the coding region of UGT1A1 in certain subjects with Gilbert’s syndrome who do not have homozygous mutations at the promoter level (Aono et al., 1995; Koivari et al., 1995; Soeda et al., 1995; Sato et al., 1998; Akaba et al., 1998, 1999; Maruo et al., 1998). The Gly71Arg mutation in the coding region has been shown to result in a 30% (heterozygotes) and 60% (homozygotes) decrease in bilirubin glucuronidating activity (Akaba et al., 1998, 1999).

UGT1A1 polymorphism plays several roles in the metabolism of irinotecan. The example of irinotecan demonstrates how a polymorphism in an inactivating metabolic pathway may affect the therapeutic outcome in cancer chemotherapy. Irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is a camptothecin derivative utilized in the treatment of metastatic colorectal cancer. Irinotecan is a prodrug, since it needs to be activated by systemic carboxylesterases to SN-38 (7-ethyl-10-hydroxycamptothecin) in order to exert its antitumor activity mediated by the inhibition of topoisomerase 1 (Fig. 2) (Kawato et al., 1991). SN-38 undergoes glucuronide conjugation to form the inactive SN-38 glucuronide (SN-38G; 10-O-glucuronoyl-SN-38). In addition, two oxidated metabolites of irinotecan have been identified as APC (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) and NPC (7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin) formed by CYP3A4 enzyme (Fig. 2). APC and NPC have shown weak antitumor activity in vitro (Rivory et al., 1996; Dodds et al., 1998).

SN-38 has been associated with the severe diarrheal episodes occurring after irinotecan therapy as a result of the direct enteric injury caused by SN-38 (Araki et al., 1993). Because it undergoes significant biliary excretion (Atsumi et al., 1991), SN-38 may potentially continue to remain in the gastrointestinal tract, resulting in prolonged diarrhea. The glucuronidation of SN-38 to the inactive SN-38G may protect against irinotecan-induced intestinal toxicities as a result of renal elimination of the more polar SN-38G (Gupta et al., 1994). Iyer et al. (1998) demonstrated the role of the UGT1A1 isoform in the glucuronidation of SN-38. Significant variability of UGT1A1 activity was evident in human livers, with a 17-fold difference between minimum and maximum rates of SN-38 glucuronidator phenotype (Iyer et al., 1999b). These findings have led to efforts directed toward the identification of innovative approaches to both predict and monitor clinical toxicity induced by irinotecan.

The assessment of pharmacodynamics of SN-38 glucuronidation showed that, with respect to the total irinotecan available in the circulation, patients with relatively low glucuronidation rates had progressive accumulation of SN-38 leading to toxicity (Gupta et al., 1994). A genetic predisposition to the metabolism of irinotecan may be critical in patients with reduced UGT1A1 activity (Iyer et al., 1998). As the distinction between mild instances of the syndrome and normal condition is sometimes difficult, Gilbert’s syndrome remains often undiagnosed. The impaired glucuronidation of SN-38 in two patients with Gilbert’s syndrome was responsible for severe neutropenia and/or diarrhea (Wasserman et al., 1997), associated with abnormally high biliary indexes, a measure of elimination of SN-38 in the bile (Gupta et al., 1994). Because of the increased risk of toxicity in genetically predisposed patients, methods more reliable than serum bilirubin levels for the assessment of UGT1A1 activity should be used before irinotecan administration. Phenotyping of patients with acetaminophen proved to be a poor predictor of SN-38 glucuronide conjugation in cancer patients (Gupta et al., 1997), probably because acetaminophen is mainly metabolized by UGT1A6 (Bock et al., 1993).

While laboratory research efforts are ongoing in our institution to identify specific UGT1A1 substrates for phenotyping purposes, the genotyping of UGT1A1 promoter mutations may predict the functional activity of UGT1A1. A correlation analysis with the corresponding phenotyping results is necessary to demonstrate the validity of the genotyping procedure. Iyer et al. (1999b) recently showed a good concordance between UGT1A1 promoter genotype and in vitro glucuronidation of SN-38 in human livers of Caucasian origin. SN-38 glucuronidation rates were significantly lower in homozygotes (TA)7/ (TA)7 and heterozygotes (TA)6/(TA)7, when compared with the wild-type genotype (TA)6/(TA)6 (Iyer et al., 1999b).

A high variability in SN-38 glucuronidation reported in liver samples from populations of African descent (Iyer et al., 1999a) can be explained by the presence of five and eight TA repeats, i.e., (TA)5 and (TA)8, in the UGT1A1 promoter (Beutler et al., 1998; DiRienzo et al., 1998). According to this evidence, greater and lesser glucuronidating activity of SN-38 has been found in (TA)5 and (TA)8 liver samples, respectively (Iyer et al., 1999a). UGT1A1 activity is inversely related to the number of TA repeats, since the transcriptional activity of the promoter decreases with the progressive increase in the number of TA repeats (Beutler et al., 1998). These new alleles indicate that up to 10 genotypes may exist at the TATAA element, probably resulting in different phenotypes with regard to bilirubin conjugation and irinotecan pharmacokinetics. Based upon in vitro phenotyping of UGT1A1 activity in livers, homozygotes for (TA)7 and heterozygotes (TA)5/ (TA)7 might be expected to have at least a 50 and 25% decrease in SN-38 glucuronidating activity, respectively (Iyer et al., 1999b). A significantly impaired ability to glucuronidate SN-38 has been found in one patient genotyped as (TA)7, homozygote (Ando et al., 1998). Consequently, appropriate irinotecan dose reductions may be necessary in homozygotes for (TA)7 and heterozygotes (TA)5/ (TA)7.

The importance of pharmacogenetic studies in cancer chemotherapy stems from the demonstration of the predictive value of a specific genotype for the pharmacodynamic effect of the treatment. With this purpose, a clinical trial at the University of Chicago is ongoing to determine the relationship between UGT1A1 promoter genotypes and...
irinotecan pharmacokinetics and gastrointestinal toxicity in cancer patients. Preliminary results suggest that, in comparison with patients with mutated alleles, patients with normal allele (TA)\(n\)/(TA)\(n\) produced SN-38 glucuronide levels at a higher extent, experiencing grade I diarrhea (Iyera et al., 2000). Patients with the (TA)\(n\) mutation developed more severe diarrhea, without significant differences between homozygotes for (TA)\(n\) and heterozygotes (TA)\(n\)/(TA)\(n\). To differentiate between homozygotes and heterozygotes for the (TA)\(n\) mutation, the actual irinotecan dose of 300 mg/m\(^2\) has been increased to 350 mg/m\(^2\), the FDA approved dose of irinotecan.

Recently, a genetic variation in the regulatory region of CYP3A4 has been discovered (Rebeck et al., 1998; Westlund et al., 1999). This variation involves an A→G substitution in the nifedipine-specific element at −287 to −296 base pairs in the regulatory region of CYP3A4 (Rebeck et al., 1998). This mutation, observed in 53% of African American and 0% in Taiwanese populations, has been shown to be associated with a 100% protection from epipodophyllotoxin-induced leukemia (Felix et al., 1998). The role of this newly discovered polymorphism in CYP3A4 in the disposition, response, and toxicity of irinotecan is not known at this time. However, this information may be critical, as the CYP3A4-mediated formation of APC is a major detoxifying pathway for irinotecan.

**Summary**

Researchers involved in drug development and clinical pharmacology are especially interested in the determination and prediction of metabolic polymorphisms to better understand and discuss individual drug responses. The determination of pharmacogenetic effects on a new drug should start as early as possible. Although the knowledge about metabolic polymorphisms has increased rapidly, the applications within the pharmaceutical industry are still limited. The results of an inquiry of the world’s largest pharmaceutical companies show that 60% of the companies had not carried out any in vitro pharmacogenetic studies at all, at the time of survey (Ball, 1997). With the development of newer methods of molecular analysis to detect DNA polymorphisms, genotyping screening approaches are increasingly becoming feasible. The use of genotyping is particularly useful when phenotyping procedures with test drugs are not yet available, as evidenced by the example of irinotecan. Individualized dosing based upon phenotype and/or genotype of relevant drug-metabolizing enzymes may improve the therapeutic index of chemotherapeutic drugs (Boddy and Ratain, 1997). Such methods have already proven to be beneficial, as seen from the studies with amonafide (Ratain et al., 1996) and 6-mercaptopurine (Lennard, 1992). The experience with amonafide and irinotecan suggests that once the functional significance of a polymorphic metabolic pathway has been established, anticancer agents are potential candidates for dose optimization by means of pharmacogenetic approaches.

**References**


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