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Severe or lethal toxicities with nucleosidic analogs: time for action

“Owing to the number of patients with cancer likely to receive these drugs as part of their treatment, implementing routine CDA testing as a means to limit drug-induced toxicities sounds like a reasonable option to ensure better safety when handling nucleosidic analogs.”

KEYWORDS: capecitabine ■ CDA ■ cytidine deaminase ■ gemcitabine ■ nucleosidic analogs ■ pharmacogenetics ■ toxicity

Nucleosidic analogs in the era of pharmacogenetics

Nucleosidic analogs are mainstays for treating several hematological malignant disorders and solid tumors, both in children and in adults. As for most cytotoxics, the therapeutic window of nucleosidic analogs is narrow and interindividual variability in their pharmacokinetic profiles may lead to life-threatening toxicities or treatment failure. Identifying genetic markers associated with response or toxicities with anticancer drugs is a major trend in clinical oncology. Beside molecular determinants at the tumor level, constitutional genetics can help to forecast clinical outcome related to changes in drug-metabolizing enzymes of anticancer agents. Changes in pharmacokinetics and subsequently in drug exposure are often driven by genetic polymorphisms affecting metabolizing enzymes in the liver, such as the canonical DPD-deficiency syndrome in patients administered 5-fluorouracil [1].

“The drawback of this single key enzymatic catabolic pathway is that subjects harboring CDA deficiency are at risk for drug overexposure with severe adverse reactions.”

Disposition of nucleosidic analogs such as gemcitabine, cytarabine, azacytidine or capecitabine is primarily dependent upon a liver catabolic or anabolic biotransformation by CDA, a ubiquitous enzyme affected by several genetic polymorphisms [2]. Toxicity of nucleosidic analogs is usually moderate when compared with other anticancer drugs; despite this, some patients display unpredictable adverse events revealing the possibility of a specific pharmacogenetic syndrome that needs to be addressed [3–5].

Integrating pharmacogenetics into gemcitabine dosing

Gemcitabine (2',2'-difluorodeoxycytidine) is a major anticancer drug acting as an antimetabolite with a growing use in solid tumors such as lung, pancreatic or gynecological cancer as part of various innovative chemotherapy protocols (e.g., Gemox and Gemcap regimens). Gemcitabine exerts its antiproliferative activity after cellular uptake and nucleosidic phosphorylation, yielding active metabolites responsible for DNA synthesis inhibition. The catabolic pathway of gemcitabine depends upon a deamination step performed by CDA leading to the inactive metabolite 2',2'-difluorodeoxyuridine. The vast majority of the administered drug dose (~80%) is inactivated through this limiting step and secondarily recovered as 2',2'-difluorodeoxyuridine in urine. The drawback of this single key enzymatic catabolic pathway is that subjects harboring CDA deficiency are at risk for drug overexposure with severe adverse reactions. Recently, several studies have focused on severe toxicities in patients treated with gemcitabine [3–5].

It has been hypothesized that impaired liver detoxification could explain the occurrence of life-threatening toxicities in patients with CDA deficiency. Approximately 25 SNPs were reported in the Single Nucleotide Polymorphism Database (dbSNP) to occur in the coding sequence of CDA, and more than 650 SNPs in the gene region. Although the genotype-to-phenotype correlation of CDA is not yet fully elucidated, several polymorphisms (e.g., 79A>C, 208G>A, -del31C and 435T>C, to name but a few) have been identified and sometimes associated with higher incidence of severe toxicities in patients upon gemcitabine intake [4,6–8].

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Alternatively, the quantification of CDA activity in serum based on a spectrophotometric assay as a surrogate marker was proposed as a standard pretreatment screening [9,10]. The 208G>A SNP has been first evidenced in a Japanese patient showing extremely severe toxicities upon gemcitabine administration [4]. Another case report demonstrated that CDA deficiency in relation to the c.246 + 37G>A mutation could be the cause of a case of toxic death in a patient treated with the gemcitabine–carboplatin combination [11]. Another study has concluded that the 79A>C polymorphism was predictive of both toxicity and efficacy in patients treated with gemcitabine-based therapy for lung cancer [12].

The role that CDA deficiency could play in gemcitabine use has been further evidenced in another study showing that CDA activity in serum below 1.4 U/mg, a value found in 10–15% of patients, was associated with the risk for developing early severe toxicities in any gemcitabine-containing regimen [9].

“Patients experienced severe and eventually unrecoverable toxicities after being administered standard [capecitabine] dosage.”

Overall and despite remaining issues with understanding genotype–phenotype relationships with CDA, there is a rising number of clinical evidence strongly suggesting that gemcitabine administration could be rendered safer by sorting patients according to their CDA status prior to starting the treatment. Beside the issue of preventing gemcitabine-related toxicities in patients with CDA deficiency, a clinical report has shown that ultrarapid-metabolizer ‘rapid-deaminator’ patients, a status found in approximately 20% of the population, could conversely be at risk of treatment failure with gemcitabine, as suggested by a preliminary report conducted in patients with pancreatic cancer [13].

The rising concern regarding dysregulation affecting CDA in gemcitabine-treated patients has prompted several groups to further study the role this polymorphic enzyme could play in the clinical outcome with other key nucleosidic analogs that are substrates for CDA too [14,15]. Some recent clinical reports have suggested that CDA deficiency could be at the origin of a severe toxicity in a young patient treated with a cytarabine-based regimen [16], and could also be linked to lethal toxicities with azacytidine [17].

Incidence of CDA ultrarapid-metabolizer status in capecitabine-based chemotherapy

Capecitabine is an oral prodrug following a three-step biotransformation pathway at the liver and tumor levels by successive action of carboxyl esterase, CDA and thymidine phosphorylase to yield 5-fluorouracil. Conversely to gemcitabine, CDA is implicated in the activation pattern of oral capecitabine, and patients with ultrarapid-metabolizer status could thus be at risk of overactivating the prodrug to 5-fluorouracil, eventually leading to severe toxicities. This has been first evidenced in a couple of patients undergoing capecitabine-based therapy [18,19]. Patients experienced severe and eventually unrecoverable toxicities after being administered standard dosage. In both cases, CDA activity proved to be markedly higher as compared with other patients, thus supporting the hypothesis that ultrarapid deamination could explain the cases.

Caronia *et al.* investigated the relevance of various polymorphisms affecting key enzymes of the capecitabine metabolism pathway as putative prognostic markers for treatment-related severe toxicities. This candidate-gene approach concluded that severe hand–foot syndrome upon capecitabine intake could be attributed to the rs3215400 SNP across the *CDA* gene promoter (-31delC). The C insertion was found to be associated with enhanced transcription by creating a binding site for the transcriptional regulator E2F [19]. Extensive phenotype in patients bearing this polymorphism could explain the higher incidence of hand and foot syndrome, although no data on enzymatic activities were made available.

At our institutes, in 2009 we demonstrated the first severe toxicity case upon capecitabine intake in a female patient with such an extensive phenotype for CDA. Although no polymorphism was identified, functional screening for CDA activity demonstrated that this patient displayed an enzymatic activity 180% higher than the mean values usually recorded in cancer patients, thus demonstrating that extensive CDA could be a condition associated with severe toxicities in capecitabine-treated patients [18]. Although there is no doubt about the existence of ultrarapid-metabolizer patients harboring enhanced CDA activity, the genetic basis for this abnormality remains unclear.

Indeed, the allelic frequency of rs3215400 is high (0.564 vs 0.436 for the C allele) suggesting that this SNP alone does not systematically lead to CDA overexpression, a phenotype observed

in less than 20% of our patients [9,13]. This suggests that no single SNP entirely accounts for observed variation in CDA activity in patients and that haplotype analysis might be more powerful to predict CDA transcription, as suggested previously in functional studies [20].

Future perspective

Despite conflicting genotype-to-phenotype relationships, a variety of clinical reports have demonstrated, either on a genotypic or a phenotypic basis, that deregulated CDA could be a culprit for severe and sometimes lethal toxicities with nucleosidic analogs. Overall, CDA status seems to be a critical condition associated with the clinical outcome of major anticancer agents such as gemcitabine and capecitabine. Although smaller to date, clinical evidence exists regarding a possible impact with cytarabine and azacytidine too. Owing to the number of patients with cancer likely to receive these drugs as part of their treatment, implementing routine CDA testing as a means to limit drug-induced toxicities sounds like a reasonable option to ensure better safety when handling nucleosidic analogs. Many genetic and epigenetic events on CDA

genes are likely to interfere with the genotype-to-phenotype relationships, as some of these regulations have yet to be discovered. However, we do think that the time for action has come, since the quantification of CDA activity in serum could be easily used as a surrogate marker, as several phenotype-based methods have been made available in the literature. Larger prospective clinical trials are currently being undertaken to untangle the role of genetic variations in CDA deficiency. These studies should also address the question of the right dose since the next step forward after identification of patients at risk will be dosing guidance and personalized prescription.

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