Received: 22 January 2019 | Revised: 28 February 2019 | Accepted: 8 March 2019

DOI: 10.1111/bcp.13921

COMMENTARY

Can cytidine deaminase be used as predictive biomarker for gemcitabine toxicity and response?

Cytidine deaminase (CDA) plays an important role in the degradation of cytidine analogues,[1] such as gemcitabine, cytarabine (ara-C), azacytidine, and aza-2′-deoxycytidine, which are widely used for the treatment of several solid and haematological malignancies. Moreover, it activates capecitabine metabolism resulting in 5-fluorouracil (5FU). CDA plays an important role in the pharmacokinetics of these analogues; Yonemori et al[2] demonstrated that a deficiency of CDA due to a specific polymorphisms would lead to excessive toxicity, while Bengala et al[3] demonstrated that both a high expression and activity of CDA in blood cells were associated with an increased degradation of gemcitabine and a lower response rate in patients with pancreatic cancer. Briefly, a lower CDA activity will lead to a decreased gemcitabine deamination to 2′, 2′-difluoro-2′-deoxyuridine (dFdU), resulting in increased plasma gemcitabine concentrations in these patients.[1-3] Thus, systemic CDA is critical for the therapeutic efficacy of gemcitabine. CDA was determined by evaluation of its gene expression and activity in white blood cells, but also by genetic polymorphisms. Certain polymorphisms (such as CDA Lys27Gln) are associated with a decreased red blood cell CDA activity,[4] but there was a large overlap in enzyme activities. In their paper, Cohen et al[5] showed that the c.-33_-31delC SNP contributed only 4.1% to the variation in CDA activity. Although significant, this does not seem relevant considering the much larger contribution of, eg, neutrophil count (24.8%). These data raised the question which assay should be used to determine CDA in patients: a genotypic assay such as SNPs or gene expression, or a phenotypic assay such as an activity assay in a blood compartment. Since cytidine analogues are widely used, a simple test would be preferred. Moreover, inflammation results in a large variation of CDA activity, leading to the hypothesis that CDA may be a marker of rheumatoid arthritis.[6] Because the variation induced by inflammation may be larger than that caused by a genotype, a phenotypic assay measuring enzyme activity to evaluate fluctuations in CDA activity would be preferable over a genomic assay. Cohen et al[5] described an independent cross-validation of a phenotypic assay, earlier developed by Ciccolini et al.[7]

CDA activity can be measured using several methods. The natural substrates for CDA are cytidine and deoxycytidine, while many cytidine analogues are excellent substrates as well, although they show different enzyme kinetics. Classical assays used a radioactive substrate, which in our hands gave similar results as HPLC assays. However, both types of assays cannot always be performed since radioactive assays require certified laboratories and HPLC assays specific equipment. Therefore, Ciccolini et al[7] developed a simple 96-well–based assay using cytidine as a substrate, which is suitable for laboratories with standard equipment including a 96-well plate reader. The assay was predictable for toxicity and was cross-validated between several laboratories within an EORTC-PAMM (Pharmacology and Molecular Mechanism) group collaboration by comparing different blood compartments and exchange of samples.[8] From these studies, it became clear that the type of the assay, the substrate, the source of the enzyme, and quantification of the protein are of ultimate importance.[8]

The strength of the paper by Cohen et al[5] is that they applied the simplified CDA assay outside the EORTC-PAMM framework and investigated a number of additional potential variables. The data confirmed the robustness of the assay in an independent laboratory, and they observed a similar variation in CDA enzyme activity, as described earlier through the Marseille-Amsterdam-Nice collaboration.[1,8] This is an important finding since in principle, this qualifies the CDA assay for a general application to select patients with either an overexpression or deletion of CDA. Recently, Tibaldi et al[9] applied the HPLC assay (using gemcitabine as a substrate) to prospectively validate CDA as a marker for gemcitabine's efficacy in patients with non–small-cell lung cancer treated with a gemcitabine combination. In that paper, the HPLC assay was compared again to the 96-well CDA assay resulting in similar conclusions.

The Cohen paper[5] also identified a number of important aspects which need validation in a larger prospective study. They demonstrated the importance of malnutrition and of inflammation by showing a relation with neutrophil count in samples of patients, before and during treatment. This aspect needs to be investigated in more detail since many patients receiving gemcitabine therapy as well other cytidine analogues (including capecitabine, which is activated by CDA) suffer from haematological toxicity and various effects on haematopoietic subpopulations, which may have different CDA activities. This means that pretreatment levels of CDA will be helpful to determine the starting dose, but monitoring CDA activity during treatment may also help to determine whether the dose of the cytidine analogues should

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2019 The Authors. British Journal of Clinical Pharmacology published by John Wiley & Sons Ltd on behalf of British Pharmacological Society.

be increased or decreased. It is not clear from their paper whether the increased CDA activity is due to differences in CDA activity in various haematopoietic subpopulations, such as an increased proportion of young immature erythroblasts. It is also not clear whether the CDA activity in the different haematopoietic lineages varies. This is an important feature which should be addressed to in future studies using CDA as a tool in selecting the correct dose of the cytidine analogue.

How to proceed with CDA testing? When taking all the data together, it is obvious that a low CDA activity is associated with altered gemcitabine pharmacokinetics (increased exposure), resulting in a high toxicity of cytidine analogues. Toxic deaths have been observed for both gemcitabine and ara-C. This pattern seems similar to dihydropyrimidine dehydrogenase (DPD, *DPYD*) testing for patients treated with 5FU or capecitabine. The first evidence for a relationship between DPD and 5FU toxicity was published more than 30 years ago, and it was suggested by us and others in the previous century that DPD testing would enable the selection of DPD deficient patients which should then either receive a lower dose or an alternative. Although the direct DPD activity assay is too complicated to be used on a large scale, a surrogate phenotyping test (ie, monitoring of plasma uracil, the natural substrate for DPD, and/or the uracil/dihydrouracil ratio) could meet this requirement. In addition, a recent prospective study combining several *DPYD* genotypes enabled the selection of patients at risk for increased toxicity, to reduce the dose of 5FU or capecitabine.[10] Since a phenotypic activity assay is more accurate in predictive drug metabolism, we propose here that a phenotypic study should be performed in which CDA activity is prospectively being determined before and during therapy (eg, 1 week before each new cycle of gemcitabine) with gemcitabine or ara-C (and other cytidine analogues, which will require a different cut-off point). Patients with an activity below the cut-off level should then be treated with a lower gemcitabine or ara-C dose, depending on the CDA activity. The study by Cohen et al demonstrates that assaying CDA at different laboratories is robust and can be performed on a timely basis.

COMPETING INTERESTS

There are no competing interests to declare.

Keywords

cytidine deaminase, gemcitabine, therapeutic drug monitoring

ORCID

Godefridus J. Peters https://orcid.org/0000-0002-5447-2877

Godefridus J. Peters[1]
Elisa Giovannetti[1,2]
Richard J. Honeywell[1]
Joseph Ciccolini[3]

[1]*Laboratory Medical Oncology, Amsterdam University Medical Center, location VUmc, Cancer Center Amsterdam, Amsterdam, The Netherlands*
[2]*Cancer Pharmacology Lab, AIRC Start-Up Unit, University of Pisa, Pisa, Italy*
[3]*SMARTc Unit, CRCM Inserm U1068 Aix Marseille Université, and La Timone University Hospital of Marseille, Marseille, France*

Correspondence
Godefridus J. Peters, Laboratory Medical Oncology, Amsterdam University Medical Center, location VUmc, Cancer Center Amsterdam, PO Box 7057, 1007 MB Amsterdam, The Netherlands.
Email: gj.peters@vumc.nl

REFERENCES

1. Ciccolini J, Serdjebi C, Peters GJ, Giovannetti E. Pharmacokinetics and pharmacogenetics of Gemcitabine as a mainstay in adult and pediatric oncology: an EORTC-PAMM perspective. *Cancer Chemother Pharmacol*. 2016;78(1):1-12.
2. Yonemori K, Ueno H, Okusaka T, et al. Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. *Clin Cancer Res*. 2005;11(7):2620-2624.
3. Bengala C, Guarneri V, Giovannetti E, et al. Prolonged fixed dose rate infusion of gemcitabine with autologous haemopoietic support in advanced pancreatic adenocarcinoma. *Br J Cancer*. 2005;93(1):35-40.
4. Giovannetti E, Laan AC, Vasile E, et al. Correlation between cytidine deaminase genotype and gemcitabine deamination in blood samples. *Nucleosides Nucleotides Nucleic Acids*. 2008;27(6-7):720-725.
5. Cohen R, Preta LH, Joste V, et al. Determination of the interindividual variability in serum cytidine deaminase activity of patients with solid tumors. *Br J Clin Pharmacol*. 2019;85(6):1227-1238.
6. Thompson PW, Jones DD, Currey HL. Cytidine deaminase activity as a measure of acute inflammation in rheumatoid arthritis. *Ann Rheum Dis*. 1986;45(1):9-14.
7. Ciccolini J, Dahan L, Andre N, et al. Cytidine deaminase residual activity in serum is a predictive marker of early severe toxicities in adults after gemcitabine-based chemotherapies. *J Clin Oncol*. 2010;28(1):160-165.
8. Peters GJ, Honeywell RJ, Maulandi M, et al. Selection of the best blood compartment to measure cytidine deaminase activity to stratify for optimal gemcitabine or cytarabine treatment. Nucleosides, Nucleotides. *Nucleic Acids*. 2014;33(4-6):403-412.
9. Tibaldi C, Camerini A, Tiseo M, et al. Cytidine deaminase enzymatic activity predicts clinical outcome in gemcitabine/platinum-treated advanced non-small-cell lung cancer patients: a multicenter prospective validation study. *Br J Cancer*. 2018;119:811-814.
10. Henricks LM, Lunenburg CATC, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol*. 2018;19(11):1459-1467.

How to cite this article: Peters GJ, Giovannetti E, Honeywell RJ, Ciccolini J. Can cytidine deaminase be used as predictive biomarker for gemcitabine toxicity and response?. *Br J Clin Pharmacol*. 2019;85:1213–1214. https://doi.org/10.1111/bcp.13921