Metabolization and Optimization of Carbocyclic 5-Aza-2′-Deoxycytidine

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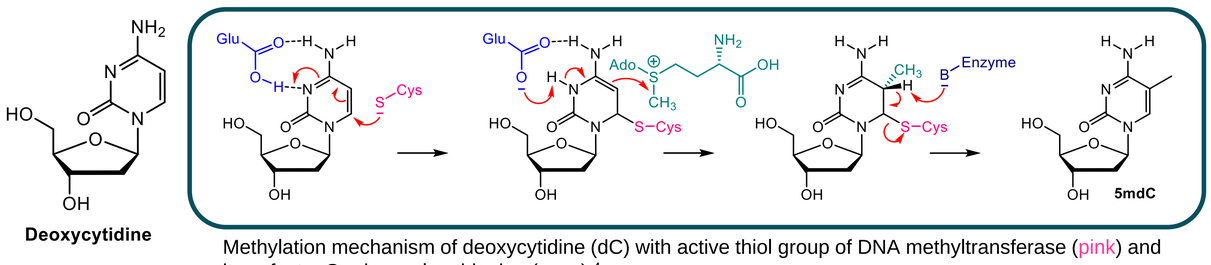
Decitabine, a DNA Methylation Inhibitor

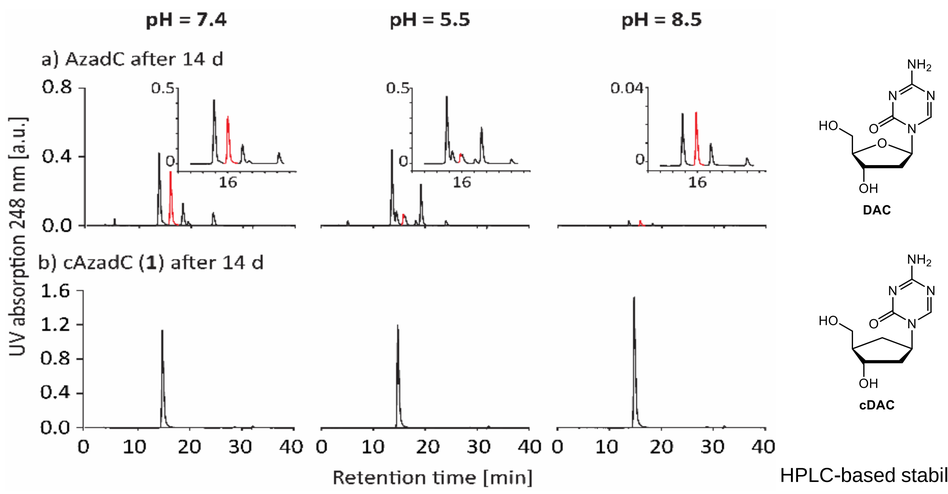
Acute myeloblastic leukaemia (AML) is a type of cancer affecting the cells of the bone marrow, preventing the normal production of blood cells. When a person is affected, the prognosis is poor. Whether due to late diagnosis or lack of more specific treatments, the cure rate is low.[1] 5-Aza-2′-Deoxycytidine (Decitabine, AzadC or DAC) is one of this drug, and like most of them it has important drawbacks. This molecule is a nucleoside analogue drug. When incorporated into DNA, it inhibits DNA methyltransferases (DNMTs) and causes the level of DNA methylation to fall, leading to the reactivation of tumour suppressor genes.[2,3] However, AzadC is used as a remedy of last resort due to significant side effects, probably because of spontaneous hydrolysis of the molecule and yet we achieved to obtain a stable derivative of this epigenetic medicine. By modifying the ribose of the base into a carbocycle (cAzadC or cDAC), we preserved the original mode of action in cellulo while obtaining a hydrolytically stable compound.[4] The current work contributes to map the overall the metabolization pathway of cAzadC in diverse cell lines and organs. The investigation of the proteins interacting with our compound allows its progressive structural optimisation to overcome potential resistance and obtain a drug for AML that is both, specific and stable.



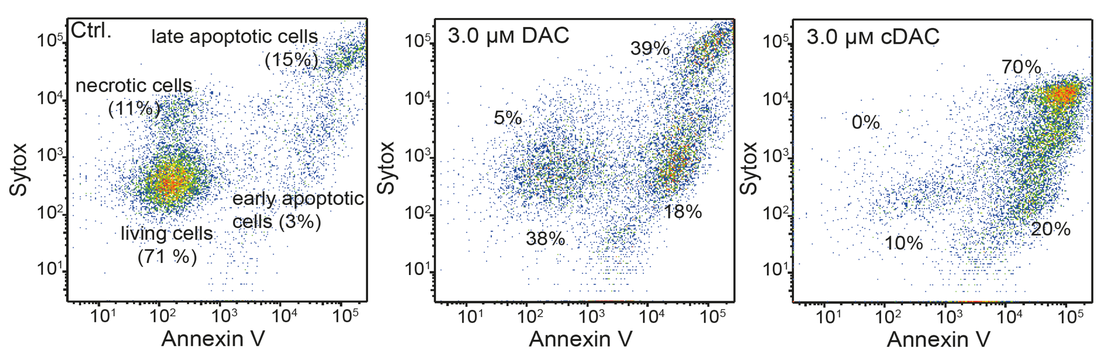
Methylation mechanism of deoxycytidine (dC) with active thiol group of DNA methyltransferase (pink) and its cofactor S-adenosylmethionine (cyan).[4]

(Upper) Methylation mechanism of 5-Aza-*2′*-deoxycytidine (AzadC) with *β*-elimination reaction blocked leading to the formation of the covalent DNA-DNMT. **(Under)** Hydrolytic degradation pathway of AzadC.

Relative Stability of AzadC vs. cAzadC



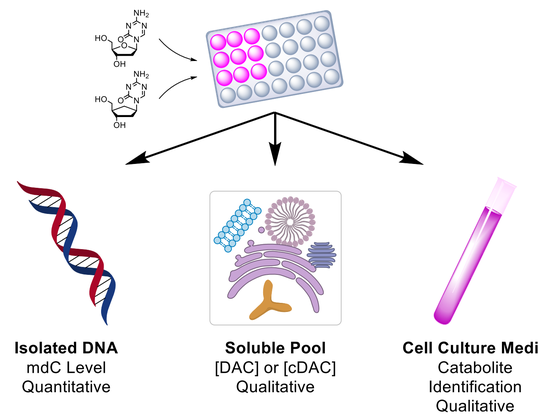
HPLC-based stability measurement.[4]



Flow cytometry, Annexin dye. Credit Dr F. Traube.

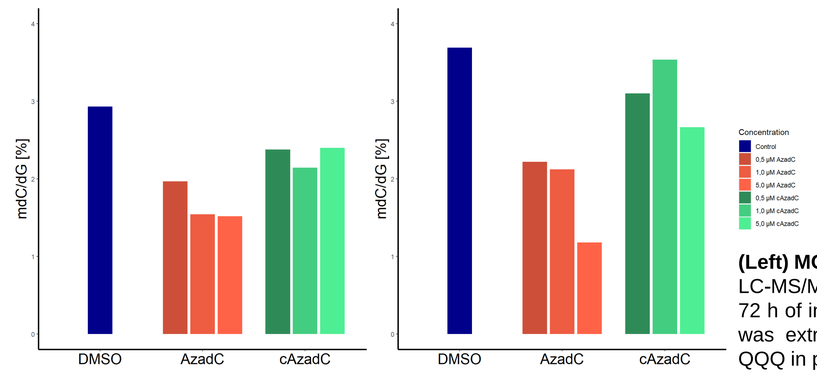
Three Steps to the Big Picture

When cells are treated with the compound of interest, we obtain three distinct sources of information. By extracting the DNA, we can quantify its methylation level. With the soluble pool, it is possible to follow the metabolization pathway of our drugs. Finally, with the media, the catabolites - products of metabolization - can be deduced.



Deoxycytidine Methylation Level, MOLM13 vs. HepG2

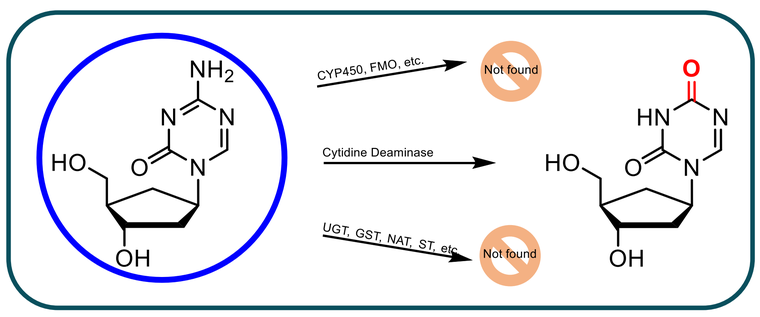
Two main cell lines are used; MOLM13 (leukaemia) and HepG2 (liver cancer). MOLM13 cells are sensitive to both AzadC and cAzadC, whereas the second cell line does not show a significant decrease in the methylation level with the carbocyclic version.



(Left) MOLM13, (Right) HepG2
LC-MS/MS quantitative measurement. After 72 h of incubation with DAC or cDAC, DNA was extracted, digest and quantified with QQQ in presence of heavy label .

Investigation of Catabolites

The metabolization of xenobiotics can be summarised as a three-phase process: I. Functionalisation, II. Conjugation III. Expulsion. By analysing the media, expulsed products can be visualised and the proteins involved in the metabolization deduced.



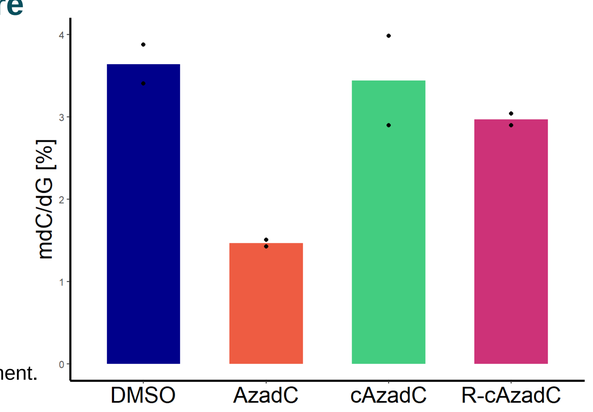
The deaminase product has been identified. Anticipated as most abundant modification: the method should be improved.

LC-MS/MS qualitative measurement. After 72 h of incubation with cDAC cell culture media was collected, treated and analyze by orbitrap.

Upgrading cAzadC Structure

The evidences on solubility and DNA incorporation issues allow us to already test cAzadC variants (R-cAzadC).

Our first In Cellulo results show a slight improvement in the reduction of mdC level.



HepG2.
QQQ quantitative measurement.

Outlook

Gradually, a better understanding of the uptake and mode of action of cAzadC in animals and in different cell lines is leading to improvements in the structure of carbocyclic aza-deoxycytidine and to a more potent drug. However, the breakthrough would be to develop a deaminase-resistant cytidine analogue drug.

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