Cellular Pharmacology of Nucleoside Analogues

IMPORTANT: Client-specified alterations can be accommodated.

Aims: Identification and quantification of a nucleoside analogue drug and its derivatives produced by the cell metabolism.
Our cellular pharmacology service is routinely performed on cultured cell extract but the analysis of nucleotide analogue and derivatives can also be done, for instance for pharmacokinetics studies, on other biological samples depending on the feasibility of the analysis.

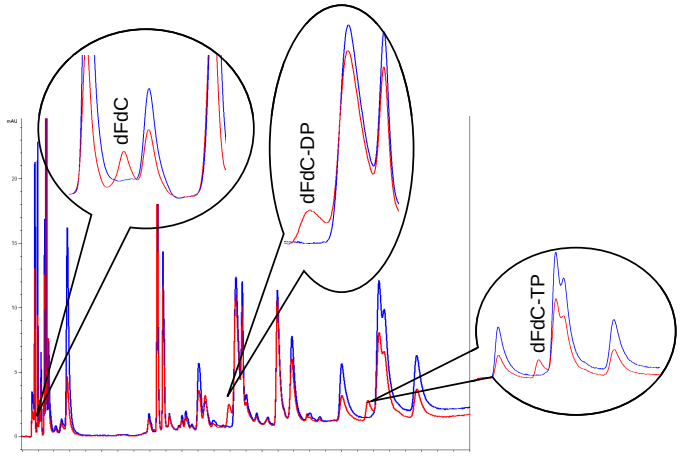
Cell permeability, target specificity and drug metabolism are crucial cellular parameters upon which the drug efficiency depends. The cell metabolism of a drug is of major importance in the case of nucleoside analogues since most, if not all, of them act as prodrugs. To be active, once entered the cell, nucleoside analogues have to be phosphorylated by cell kinases. Mono-, di- and tri-phosphate nucleotide forms, as well as other possible cell-modified derivatives (*e.g.* deamination) of the corresponding nucleoside analogue act differently on the cell metabolism. (*see references*).

Nucleotides and nucleosides Analysis: The separation and analytical procedures developed by **NOVOCIB** are particularly relevant to study the cellular pharmacology of nucleoside analogues. They have been optimized for several nucleoside analogues, particularly with ribavirin. However, the separation and analytical procedures must be specifically adapted to every nucleoside analogue.

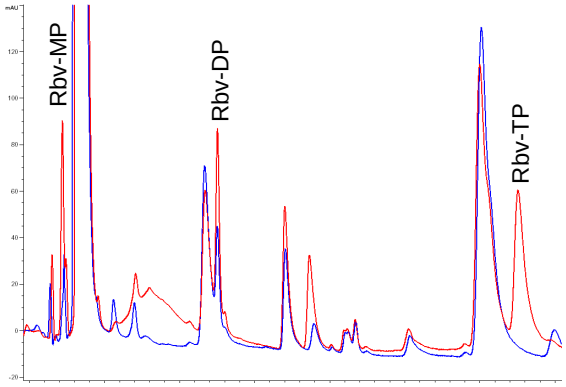
Cell culture and treatment: The choice of the cell line and culture conditions has been optimized to get highly reproducible results. Assays are usually done with human hepatoma cell line Huh7. Cells are grown in DMEM supplemented with FCS (5%), glutamine (1mM), sodium pyruvate (1mM) and maintained in exponential phase. Cells are seeded on 10cm-dishes and allowed to adhere overnight. The drug is added next day at the agreed concentration and at a cell confluence of about 50%.
Other samples than cultured cells can be analyzed: blood cells, body fluid...

Do not hesitate to **contact us** for any matter of feasibility!

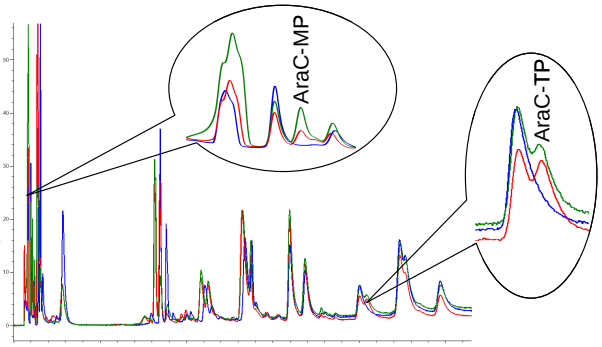
*Separation of gemcitabine (dFdC) and gemcitabine di- (dFdC-DP) and triphosphate (dFdC-TP) in Huh7 cell extract *:*
Blue: non-treated cells
Red: 48h-treatment with gemcitabine (dFdC) at 200 µM
** dFdC-MP was not detected in this experiment*



*Separation of ribavirin (Rbv) and ribavirin mono- (Rbv-MP), di- (Rbv-DP) and triphosphate (Rbv-TP) in Huh7 cell extract *:*
Blue: non-treated cells
Red: 48h-treatment with ribavirin at 3 mM



Separation of cytarabine (AraC) and cytarabine mono- (AraC-MP), di- (AraC-DP) and triphosphate (AraC-TP) in Huh7 cell extract:
Blue: non-treated cells
Red: 48h-treatment with cytarabine (AraC) at 130 µM
Green: 48h-treatment with cytarabine (AraC) at 260 µM



References
R. G. Gish (January 2006): **Treating HCV with ribavirin analogues and ribavirin-like molecules** *J. Antimicrob. Chemother.* 57(1), 8–13
E. Mini, S. Nobili, B. Caciagli, I. Landini & T. Mazzei (May 2006): **Cellular pharmacology of gemcitabine** *Ann. Oncol.* 17 (5), v7-v12
J. Z. Wu, C.-C. Lin and Z. Hong (October 2003) **Ribavirin, viramidine and adenosine-deaminase-catalysed drug activation: implication for nucleoside prodrug design** *J. Antimicrob. Chemother.* 52(4), 543-546
S. Madajewicz, P. Hentschel, P. Burns, R. Caruso, J. Fiore, M. Fried, H. Malhotra, S. Ostrow, S. Sugarman, and M. Viola (October 2000): **Phase I chemotherapy study of biochemical modulation of folinic acid and fluorouracil by gemcitabine in patients with solid tumor malignancies** *J. Clin. Oncol.* 18(20), 3553-3557