Inhibition of DNA methyltransferase by RX-3117 (fluorocyclopentenylcytosine) leads to upregulation of hypomethylated targets

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INTRODUCTION

• RX-3117 (fluorocyclopentenylcytosine) is a novel cytidine analogue
• RX-3117 resembles azacytidine (aza-CR) and aza–deoxyctydine (aza-CdR)
• RX-3117 is incorporated into RNA and DNA
• RX-3117 is active in cell lines and tumors resistant to gemcitabine⁴,⁵

Aims of the Study

Does RX-3117 treatment affect:
• Expression and activity of DNMT1?
• DNA methylation?
• The function of proteins for which the gene is known to be regulated by methylation:
  - Proton-coupled folate transporter (PCFT): transports folic acid, methotrexate (MTX) and pemetrexed (PMX) at pH 5.5 and 7.4, and the gene is highly methylated⁶
  - E-cadherin, an adhesion molecule
  - p16INK4, a tumor suppressor protein
  - G6-Methylgine DNA methyletransferase (MGMT), a DNA repair gene

REFERENCES


RESULTS

RX-3117 down-regulates DNMT1 protein and gene expression

Cell lines:
- CCRF-CEM cells and its MTX resistant variant CEM-MTX, characterized by a deficiency of the reduced folate carrier (RFC): The PCFT gene in CEM cells is highly methylated⁶
- CEM cells are cultured in RPMI medium with 10% fetal bovine serum (FBS)
- A549 and SW1573 non-small cell lung cancer (NSCLC) and A2780 ovarian cancer cell lines, which are cultured in DMEM medium with 10% FBS

Western Blots, immunohistochemistry and RT-PCR

- DNMT1 protein expression was measured by Western Blotting after exposure to RX-3117 for 24 or 48 hr
- DNA methylation was measured by real time PCR after 24 and 48 hr exposure to RX-3117
- DNMT enzyme activity was measured in isolated nuclei after exposure 1 µM RX-3117 or 5 µM aza-CdR using a DNA methyltransferase assay kit provided by Epigentek using the ability of a CpG binding domain to bind to methylated DNA.
- In A549 cells the effect of 5 µM RX-3117 on overall methylation was measured with a specific antibody against 5-methyl cytosine
- Bands on Western blots were visualized using appropriate InfraRedDye using an Odyssey Infrared imager

DNA methyletransferase (MTX) transport

MTX transport was measured using radiolabeled MTX in CEM wild type and CEM-MTX cell lines.
- CEM cells have a high RFC activity: CEM-MTX are completely deficient in RFC-mediated transport
- CEM cells have a highly methylated PCTT transporter and a very low RFC-mediated transport⁷
- MTX transport at pH 7.4 is predominantly RFC mediated and less than 2% by PCFT
- Folic acid was used to inhibit RFC mediated transport
- L-leucovorin (LV) was added to completely inhibit RFC mediated transport
- CEM and CEM-MTX cells were exposed to 50.6 µM RX-3117 and to 0.19 µM aza-CdR as a positive control
- MTX transport was measured after 24 hr to the drugs in a 3 minutes uptake assay using 2 µM (3,5,7,9H)MTX.

CONCLUSIONS

- RX-3117 downregulates DNMT1 protein and RNA expression
- RX-3117 decreases DNA methylation
- RX-3117 mediated hypomethylation increases:
  • expression of MGMT and E-cadherin
  • PCFT mediated transport of MTX
RX-3117 is a new epigenetic modulator

Effective 24 hr exposure to RX-3117 on PCFT mediated transport of MTX. Folic acid (FA) was added to inhibit PCFT and L-LV to inhibit RFC mediated MTX transport. Aza-CdR and Aza-CL were included as a positive control.