

15th International HIV Drug Resistance Workshop



13-17 June 2006, Sitges, Spain

SELECTIVE EXCISION OF NON-OBLIGATE CHAIN-TERMINATORS BY THE HEPATITIS C VIRUS NS5B POLYMERASE

Antivir Ther. 2006, 11:S3 (abstract no. 1)

J Deval, CM D'Abramo and M Götte

McGill University, Montreal, Canada

BACKGROUND: The phosphorolytic excision of classical chain-terminators by HIV-1 reverse transcriptase (RT) is detrimental to the inhibitory effects of these compounds. It is unknown whether nucleoside analogues that inhibit RNA synthesis by the hepatitis C virus (HCV) NS5B polymerase are likewise vulnerable to excision. Here we studied the phosphorolytic excision of the non-obligate chain-terminators 2'-C-methylated adenosine (2'-C-Me-A) and 2'-C-methylated cytidine (2'-C-Me-C). A prodrug of the latter has recently advanced into clinical trials.

METHODS: We have purified wild type HCV NS5B and the S282T mutant that has been associated with resistance to 2'-modified nucleotides. Both enzymes were compared with respect to their abilities to incorporate and to excise structurally distinct chain-terminators.

RESULTS: In the presence of physiologically relevant concentrations of PPi, the HCV enzyme is capable of removing both classical and non-obligate chain-terminators from the 3' end of the primer. The S282T mutation does not increase rates of excision. Decreased susceptibility to 2'-C-methylated-nucleotides appears to be based solely on an improved discrimination between the inhibitor and its natural counterpart. Most importantly, we found that the efficiency of excision is largely influenced by the nature of the nucleobase. Pyrimidines are more efficiently excised than purines. Moreover, the presence of the next complementary nucleotide literally blocks excision of the purine 2'-C-Me-A, while the pyrimidine 2'-C-Me-C is still efficiently cleaved under these conditions. Thus, in contrast to 2'-C-Me-C, the A-analogue appears to allow binding of the next nucleotide. Nucleotide binding is not productive with regards to both the forward and the back reaction, which helps to explain why 2'-C-Me-A can act as a chain-terminator that is also protected from excision.

CONCLUSIONS: The results of this study suggest that the phosphorolytic removal of incorporated pyrimidine analogues is an important factor that can diminish the inhibitory effects of these compounds. Our findings provide a rational for the development of purine analogues as antiviral drugs, despite the fact that these compounds compete with high intracellular concentrations of ATP and GTP.

2006-06-13

1

Copyright © 2006 - [International Medical Press Ltd.](#) Reproduction of this abstract (other than one copy for personal reference) must be cleared through the International Medical Press Ltd. 2-4 Idol Lane, London EC3R 5DD UK.