103

Specific Inhibition Of Influenza Transcriptase By 2'-Deoxy -2'-Fluoroguanosine.

M. Tisdale, M. Ellis, S. Court, M. Ford. Department of Molecular Sciences, The Wellcome Research Laboratories, Beckenham, BR3 3BS , U.K..

2'-deoxy-2'-fluoroguanosine (2'-fluoro-dGuo) inhibits the replication of all influenza A and B strains tested in cell culture and in the lungs of influenza infected mice which results in increased survival of mice*.Previously we have shown that the compound is phosphorylated by cellular deoxycytidine kinase and that the triphosphate selectively inhibits the influenza transcriptase from disrupted virus+.We have extended these studies by determining the activity of 2'-fluoro-dGuo in cell culture on primary and secondary virus transcription and on virus and cell protein synthesis. In addition we have examined the potential for development of resistance with recombinant A/X31virus by serial passage in CEF cells in increasing concentrations of compound.Virus with a 5 fold shift in sensitivity to 2'-fluoro-dGuo was isolated after 10 passes. Comparative kinetic studies with the influenza transcriptase from purified parent and mutant virus revealed significant changes in both the Ki and the GTP substrate Km of the mutant virus. Sequencing of the virus transcriptase gene,PB1, has revealed at least one consistent amino acid substitution present in resistant virus. These observations indicate that 2'-fluoro-dGuo is a specific inhibitor of the influenza transcriptase which targets the active site of the PB1 polymerase protein.

REFERENCES

* Tisdale *et al.*,(1993)Antiviral Chemistry and Chemotherapy-in press

+ Tisdale *et al.*,(1992)Antiviral Research,Suppl.1 Abstr 88.

104

Molecular Characterization of a Poliovirus Enhanced Mutant Selected by the Antiviral Compound 3(2H)-isoflavene.

D. Genovese (1), S. Catone (1), M. Elmi Farah (2), A. Gambacorta (3) and L. Fiore (1).

1) Lab. of Virology, Istituto Superiore di Sanità, Rome, Italy- 2) Dept. of Chemistry, "La Sapienza" University, Rome, Italy - 3) Dept. of Chemistry, III University, Rome, Italy.

3(2H)-isoflavene is a compound, correlated to 4',6-dichloroflavan, which possesses an in vitro anti-poliovirus activity. Previous studies indicated that this compound acts during the early stages of poliovirus replication, but it does not affect binding, penetration or viral uncoating. Transfection with purified viral RNA in the presence of the compound results in the inhibition of viral growth, showing that the antiviral activity of the drug is capsid independent. The anti-poliovirus activity of this compound can be a useful tool to understand the molecular basis of viral replication. A series of spontaneous mutants which were derivatives of P712 poliovirus type 2, were selected for resistance by growing them in the presence of increasing concentrations of 3(2H)-isoflavene. When progeny was checked for resistance by plating in the presence and absence of the drug, enhanced phenotypes emerged. Plating efficiency of drug-enhanced mutants was higher than 1000-fold in the presence of the compound than in its absence. To clarify the mechanism of action of the 3(2H)-isoflavene, one of the enhanced mutants was studied at the molecular level. The results of the viral RNA sequencing will be presented.