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Influence of *S*-Adenosylhomocysteine Hydrolase Inhibitors on *S*-Adenosylhomocysteine/*S*-Adenosylmethionine Pool Levels in Murine L929 Cells

M. Cools and E. De Clercq
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

S-Adenosylhomocysteine (AdoHcy) hydrolase has been recognized as the target enzyme for the antiviral activity of several carbocyclic and acyclic adenosine analogues. In a previous study [Cools and De Clercq, Biochem. Pharmacol. 38, 1061-1067 (1989)], we have found a close correlation between the antiviral activity of six adenosine analogues [(*S*)-9-(2,3-dihydroxypropyl)adenine, (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid (isobutyl ester), 3-deazaneplanocin A, carbocyclic 3-deazaadenosine, adenosine dialdehyde and neplanocin A] against vaccinia virus and vesicular stomatitis virus and the inhibitory effect of these compounds on purified AdoHcy hydrolase isolated from murine L929 cells. We have now examined the effects of the different adenosine analogues, at concentrations which reduce vaccinia virus growth by 90% (IC_{90}), on the intracellular pool levels of AdoHcy and *S*-adenosylmethionine (AdoMet). Treatment of mock-infected and vaccinia virus-infected L929 cells for 12 hr with the adenosine analogues at their IC_{90} increased the AdoHcy levels from 0.02 nmoles/mg protein to approximately 0.30 nmoles/mg protein. No differences were observed between the AdoHcy pool levels of vaccinia virus-infected cells and mock-infected cells. The compounds did not alter the AdoMet pool levels in either mock-infected or virus-infected cells. These findings indicate that the antiviral action of the AdoHcy hydrolase inhibitors against vaccinia virus may be directly related to the raise in intracellular AdoHcy pool levels.

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Study of the Binding Parameters of R 61837 to HRV 9 and Immuno-Biochemical Evidence for a Capsid Stabilizing Activity of the Drug.

Marc Moeremans, Marc De Raeymaeker, Guy Daneels, Marc De Brabander, Koen Andries, Janssen Research Foundation, B-2340 Beerse (Belgium)

Earlier studies have suggested that the antirhinovirus agent R 61837 or 3-methoxy-6-[4-(3-methylphenyl)-1-piperazinyl]pyridazine neutralizes virus infectivity by a direct interaction with the virus capsid. In order to find more evidence for this proposed mechanism of action, drug interactions with HRV 9 (wild type) and with a semi-drug-resistant mutant (HRV 9H) were studied in more detail. Using radiolabelled drug, it was demonstrated that the drug binds to native particles only. For both strains tested, there was a good correlation between the K_D (calculated from the Scatchard plots) and the MIC values (K_D HRV 9 = 3.67E-8, MIC = 2.11E-8; K_D HRV 9H = 2.99E-7, MIC = 4.40E-7). Reversibility experiments showed that more than 60 % of the drug could be extracted with chloroform from HRV 9H but only 5 % from HRV 9. Dissociation studies demonstrated that in the presence of excess unlabelled drug the $t_{1/2}$ value for HRV 9 and HRV 9H are, respectively, 385 and 15 min. Rate zonal centrifugation experiments using ^{35}S-methionine labelled virus showed that upon binding the virus was protected against heat (56 °C) and acid (pH 5.0), although at the same drug concentration the resistant strain was stabilized to a lesser extent. These data were also confirmed immunochemically by using a neutralizing monoclonal antibody. The drug prevented the switch from D to C antigenicity which is induced by exposure of the virus to mild denaturing conditions. These data indicate that the drug is apparantly able to prevent a conformational change of the capsid which may be a prerequisite for infection.