124

Sensitivity of Mutated Herpes Simplex Virus Type-1 DNA Polymerase to Conventional Guanine and Novel Cyclobutane Guanine Nucleotide Analogues.
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Using in vitro mutagenesis, point mutations were introduced into the herpes simplex virus type-1 (HSV-1) DNA polymerase and the mutants expressed in S. cerevisiae. Mutations include single amino acid substitutions at residues 355 and 696, and a series of mutations at both residues 724 and 815. The mutated enzymes were evaluated for HSV-1 specific DNA polymerase activity and for sensitivity to aphidicolin, phosphonoacetic acid and the guanine nucleotide analogues, acyclovir and ganciclovir triphosphates (ACV-TP and GCV-TP respectively). We also compared the sensitivity of the mutants to the triphosphates of the novel, cyclobutane nucleotide analogues: (R,S)-(1α,2β,3α)-9-[2,3-bis-(hydroxymethyl)cyclobutyl]guanine, (R,S)-BHCG and (R,S)-(1α,2β,3α)-9-[2-hydroxy-3-(hydroxymethyl)cyclobutyl]guanine, (R,S)-HHCG. Substitutions at residues 724 and 815 exhibited antiviral sensitivities distinct from those at residue 696. Mutations (724 and 815) which conferred resistance to ACV-TP, did not confer cross-resistance to (R)-BHCG-TP or GCV-TP. The sensitivity of 696 to ACV-TP, (R,S)-HHCG-TP or (R)-BHCG-TP was unaltered, but resulted in hypersensitivity to GCV-TP. Our molecular model of HSV-1 DNA polymerase suggested residues 724 and 815 may be close to each other in the three dimensional structure; residue 696 is not. The difference in antiviral sensitivities reported here is consistent with this spatial separation.

125

Lithium Resistant Herpes Virus Mutant.
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Recent research has shown that the lithium ion inhibits the replication of DNA viruses, e.g. herpes simplex virus (HSV), in vitro. There is also evidence that lithium may have a role to play as a local agent for the treatment of cutaneous or muco-cutaneous infections. During studies investigating the effect of lithium succinate on the behaviour and replication of HSV-1 (strain troisbel), mutant virus strains have been isolated that are resistant to concentrations of lithium which are inhibitory to wild-type HSV-1. Resistance to lithium bred true following cloning and passage in the presence and absence of lithium. The mutant virus strains were also found to be less sensitive to acyclovir than wild-type virus. The polypeptide profiles of mutant and wild type viruses were compared by poly-acrylamide gel electrophoresis after ^{35}S methionine and ^{14}C glucosamine incorporation. In both wild-type and mutant virus strains, synthesis of the major capsid polypeptide, glycoprotein C and polypeptides of 70, 60, 48, 35,000 Mwt. were reduced in the presence of lithium. These reductions were however less marked with the lithium-resistant mutant strains particularly glycoprotein C; this was confirmed by glucosamine-labelling studies. Studies using cytosine arabinoside at concentrations which inhibit DNA synthesis indicate that the effect of lithium on polypeptide synthesis is similar to, but not as pronounced as the effect of cytosine arabinoside on polypeptide synthesis. It may be that these effects are secondary to potassium uptake as will be discussed in "The molecular effect of lithium on herpes simplex virus replication". Studies using these resistant virus strains could be an important link in determining the mode of action of lithium and possibly establishing an enzyme-binding site for Li^+.