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VACCINES AND ANTIVIRAL AGENTS

Escape of Tick-Borne Flavivirus from 2′-*C*-Methylated Nucleoside Antivirals Is Mediated by a Single Conservative Mutation in NS5 That Has a Dramatic Effect on Viral Fitness

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ABSTRACT Tick-borne encephalitis virus (TBEV) causes a severe and potentially fatal neuroinfection in humans. Despite its high medical relevance, no specific antiviral therapy is currently available. Here we demonstrate that treatment with a nucleoside analog, 7-deaza-2′-*C*-methyladenosine (7-deaza-2′-CMA), substantially improved disease outcomes, increased survival, and reduced signs of neuroinfection and viral titers in the brains of mice infected with a lethal dose of TBEV. To investigate the mechanism of action of 7-deaza-2′-CMA, two drug-resistant TBEV clones were generated and characterized. The two clones shared a signature amino acid substitution, S603T, in the viral NS5 RNA-dependent RNA polymerase (RdRp) domain. This mutation conferred resistance to various 2′-*C*-methylated nucleoside derivatives, but no cross-resistance was seen with other nucleoside analogs, such as 4′-*C*-azidocytidine and 2′-deoxy-2′-beta-hydroxy-4′-azidocytidine (RO-9187). All-atom molecular dynamics simulations revealed that the S603T RdRp mutant repels a water molecule that coordinates the position of a metal ion cofactor as 2′-*C*-methylated nucleoside analogs approach the active site. To investigate its phenotype, the S603T mutation was introduced into a recombinant TBEV strain (Oshima-IC) generated from an infectious cDNA clone and into a TBEV replicon that expresses a reporter luciferase gene (Oshima-REP-luc2A). The mutants were replication impaired, showing reduced growth and a small plaque size in mammalian cell culture and reduced levels of neuroinvasiveness and neurovirulence in rodent models. These results indicate that TBEV resistance to 2′-*C*-methylated nucleoside inhibitors is conferred by a single conservative mutation that causes a subtle atomic effect within the active site of the viral NS5 RdRp and is associated with strong attenuation of the virus.

IMPORTANCE This study found that the nucleoside analog 7-deaza-2′-*C*-methyladenosine (7-deaza-2′-CMA) has high antiviral activity against tick-borne encephalitis virus (TBEV), a pathogen that causes severe human neuroinfections in large areas of

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