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Abstract

An E460D Substitution in the NS5 Protein of Tick-Borne Encephalitis Virus Confers Resistance to the Inhibitor Galidesivir (BCX4430) and Also Attenuates the Virus in Mice †

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Abstract: Tick-borne encephalitis virus (TBEV) is a pathogen that causes severe human neuroinfections in Europe and Asia for which there is currently no specific therapy. The adenosine analogue galidesivir (BCX4430), a broad-spectrum RNA virus inhibitor, has entered a phase 1 clinical safety and pharmacokinetics study in healthy subjects and is under clinical development for treatment of Ebola and yellow fever virus infections. Moreover, galidesivir also inhibits the reproduction of TBEV and numerous other medically important flaviviruses. Until now, studies of this antiviral agent have not yielded resistant viruses. In our study, we performed serial in vitro passaging of TBEV in the presence of increasing concentrations of galidesivir (up to 50 μM), which resulted in the generation of two drug-resistant TBEV mutants. The first TBEV mutant was characterized by a single amino acid change, E460D. The other carried two amino acid changes, E460D and Y453H. Both mutations mapped to the active site of the viral RNA-dependent RNA polymerase (RdRp). Galidesivir-resistant TBEV exhibited no cross-resistance to structurally different antiviral nucleoside analogues, such as 7-deaza-2′-C-methyladenosine, 2′-C-methyladenosine, and 4′-azido-aracytidine. Although the E460D substitution led to only a subtle decrease in viral fitness in cell culture, galidesivir-resistant TBEV was highly attenuated in vivo, with a 100% survival rate and no clinical signs observed in infected mice. Furthermore, no virus was detected in the sera, spleen, or brain of mice inoculated with the galidesivir-resistant TBEV. By contrast, infection with wild-type virus resulted in fatal infections for all animals. Our results contribute to understanding the molecular basis of galidesivir antiviral activity, flavivirus resistance to nucleoside inhibitors, and the potential contribution of viral RdRp to flavivirus neurovirulence.

Keywords: BCX4430; attenuation; drug resistance; galidesivir; mutation; tick-borne encephalitis virus