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Response to Letter to the Editor

Response to Comments on "Remdesivir and EIDD-1931 Interact with Human Equilibrative Nucleoside Transporters 1 and 2: Implications for Reaching SARS-CoV-2 Viral Sanctuary Sites"

Siennah R. Miller, Meghan E. McGrath, Kimberley M. Zorn, Sean Ekins, Stephen H. Wright, and Nathan J. Cherrington

College of Pharmacy, Department of Pharmacology & Toxicology (S.R.M., M.E.M., N.J.C.) and Department of Physiology (S.H.W.), University of Arizona, Tucson, Arizona; and Collaborations Pharmaceuticals, Inc., Raleigh, North Carolina (K.M.Z., S.E.)

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We thank Dr. Eric Johnson (Johnson, 2022) for the comments regarding our recent study (Miller et al., 2021) and providing us with the opportunity to further emphasize the importance of the equilibrative nucleoside transporters (ENTs) in nucleoside analog disposition. Nucleoside analogs are an important drug class used to treat viral infections and cancer and have recently gained attention as a potential therapeutic option for the treatment of COVID-19. Remdesivir is currently used to treat COVID-19, and molnupiravir recently had a positive readout in phase 2/3 clinical trials for the treatment of COVID-19 (NCT04575597). Our study showed that remdesivir is a substrate of ENT1 and ENT2, and the active metabolite of molnupiravir (EIDD-2801) is also a substrate of these transporters (Miller et al., 2021). Alterations to transporter function and/or expression may potentially impact the therapeutic benefit of nucleoside analog drugs, which relate to the points Dr. Johnson addresses in his letter to the editor. It will also be interesting to see the clinical effectiveness of molnupiravir, given the fact that its active metabolite, EIDD-2801, is also a substrate.

As Dr. Johnson notes, ENT1 and ENT2 are downregulated in lung epithelial and endothelial cells during hypoxia and acute lung injury (Eltzschig et al., 2005; Morote-Garcia et al., 2013). HIF-1-alpha mediates the repression of ENT expression in hypoxia, and HIF-1α mRNA expression is elevated in COVID-19 (Morote-Garcia et al., 2013; Taniguchi-Ponciano et al., 2021). In addition to downregulation of ENTs, increased extracellular levels of adenosine have been observed in acute lung injury (Eckle et al., 2009). Both of these observations have the potential to limit antiviral entry into cells as high levels of extracellular adenosine may also competitively inhibit uptake of ENT substrates.

Viral infections are documented to downregulate ENT expression and impair antiviral effects of known ENT substrates, including ribavirin in hepatitis C virus infected cells (Panigrahi et al., 2015). In addition to these observations, antivirals may also be substrates of P-glycoprotein (P-gp), including remdesivir (Gilead Sciences, 2020). Efflux by P-gp, therefore limiting intracellular accumulation of drug, may also explain discrepancies between in vitro efficacy and human efficacy data if the cellular model systems used for in vitro efficacy do not express P-gp.

Future studies investigating the impact of SARS-CoV-2 infection in vitro on ENT expression have the potential to explain differences in efficacy of remdesivir, molnupiravir, and other nucleoside analogs being developed for treatment of COVID-19. ENT1 and ENT2 provide an additional entry route for this class of antivirals, which are substrates of these transporters. Our study highlights the pertinent relevance of these transporters in antiviral drug disposition and opens the door for future studies on viral infection mediated transporter regulation.

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ABBREVIATION: ENT, equilibrative nucleoside transporter; P-gp, P-glycoprotein.

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Address correspondence to: Nathan J. Cherrington, University of Arizona, 1703 E. Mabel Street, Tucson, AZ 85719. E-mail: cherrington@pharmacy.arizona.edu

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