Mechanism of action of favipiravir against SARS-CoV-2: Mutagenesis or chain termination?

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Favipiravir is a novel broad-spectrum antiviral drug against RNA viruses that has been approved for the treatment of seasonal and pandemic influenza. The use of favipiravir is a potential therapeutic approach to SARS-CoV-2 infection; several clinical trials have shown that favipiravir could effectively improve the clinical recovery and/or viral RNA clearance of patients, with proven safety profiles.[1,2] Favipiravir is a prodrug and its active form, favipiravir ribofuranosyl-triphosphate (FTP), inhibits viral replication by targeting the RNA-dependent RNA polymerase (RdRp). However, the mechanism by which FTP interacts with RdRp has not been fully elucidated.

The two main hypotheses of the action of favipiravir are: (1) FTP forms intracellularly, acts as a nucleoside analog to simulate GTP/ATP, and subsequently incorporates itself into the RNA of nascent viruses, resulting in the termination of RNA synthesis; (2) FTP incorporation into viral RNA induces high rates of genome mutation that leads to the loss of infectivity or the production of nonviable virions, further inhibiting viral replication and reproduction.

A recent study published in *The Innovation*, "Structural basis of SARS-CoV-2 polymerase inhibition by favipiravir," explored the mechanism of favipiravir in inhibiting the SARS-CoV-2.[3] The study used structural biology methods and provided a rational pharmacological design and development justifications for more effective antiviral drugs against SARS-CoV-2.

In this study, primer extension assays were performed to investigate how FTP interfered with RNA synthesis.[3] The results showed that favipiravir acted as a purine nucleotide analog to mimic the incorporation of A and G nucleotides into nascent RNA products. However, it did not significantly inhibit RNA production, suggesting that the incorporation of favipiravir is likely to induce mutations in progeny viral genome. This result is consistent with the results reported by Shannon et al.[4] Subsequently, the authors determined the high-resolution structure of the favipiravir-SARS-CoV-2 polymerase complex using cryoelectron microscopy (cryo-EM) reconstruction. It was revealed that favipiravir, which was recognized as a form of FTP, was bound at the +1 position and paired with template C residue. The structural basis study indicates a pre-catalytic conformation of SARS-CoV-2 polymerase at an earlier stage before the post-catalysis conformation, which covers an important link in the catalytic cycle of polymerase. However, another recent study corroborated the inhibition of RNA replication, which is mediated by chain termination.[5] Primer extension activity assays showed that FTP was weakly incorporated into the RNA primer strand and induced premature replication termination.[5] The structural basis study, performed using the cryo-EM method, showed that favipiravir stalls replication by noncovalent interactions at the active site rather than by covalent incorporation into the replicating strand. This reveals a nonproductive binding mode of FTP at the catalytic site of SARS-CoV-2 RdRp. Based on the controversial outcomes of these studies, the primer-template sequences and substrate supply used in the primer extension assays may contribute to the differences. In the absence of NTP, several repeated uracil residues in the template (e.g., UUUU in Peng et al.;[3] UUUUU in Naydenoya et al.[5]), which allow consecutive FTP incorporations, contributed to the strand termination; however, the situation seemed to occur a little less frequently during viral replication. With only the absence of ATP or GTP, efficient FTP incorporation and elongation occurred along the RNA template resulting in the accumulation of full-length products, indicating that lethal mutagenesis is the mechanism of action for favipiravir.[3,4] The distinct structural snapshots of the RdRp-template-primer-FTP complex captured using cryo-EM in Peng et al.[3] and Shannon et al.[4] provided a productive conformation and nonproductive conformation, respectively. These studies indicate that favipiravir inhibits viral replication predominantly by inducing mutations in the progeny genome and is different from remdesivir, which impairs the elongation of RNA production (Figure 1).

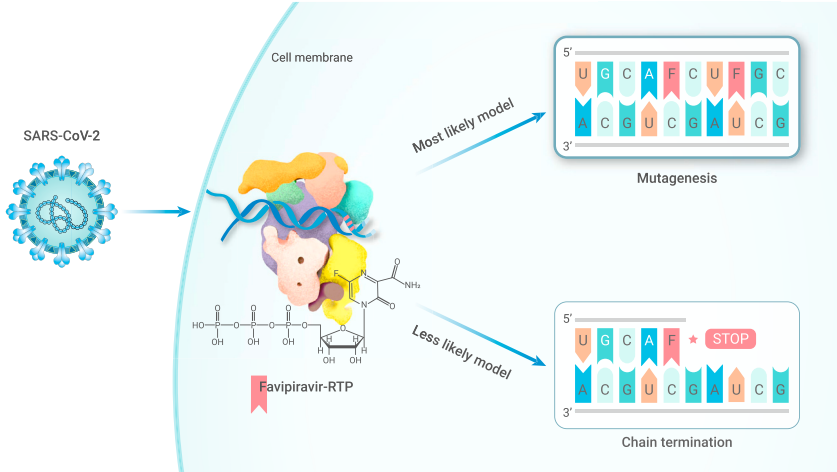

Figure 1. Mechanism of action of favipiravir against SARS-CoV-2

Favipiravir is a nucleoside analog derived from pyrazine carboxamide, which has a lower molecular complexity than remdesivir. This may be the reason for decreased destabilization of the replication complex. The effect of the penta-heterocycle structure on their interaction with polymerase contributes to the difference in the mechanism of action between favipiravir and remdesivir.

In addition, the interactions of FTP with polymerase are highly similar to those of GTP, and the recognition residue sites are conserved across many RNA viruses. This suggests that favipiravir may have broad-spectrum antiviral activity against various RNA viruses through similar mechanisms. These findings further reveal the catalytic mechanism of polymerase and provide a structural basis for further understanding of the mechanism of favipiravir against SARS-CoV-2.

Efficacy, affordability, and ease of access (e.g., oral route) of antiviral drugs are essential for the prevention and control of epidemic situations caused by viruses such as SARS-CoV-2. One of the most promising strategies to develop broad-spectrum antiviral drugs is by targeting the highly conserved RdRp structure. Several inhibitors of SARS-CoV-2 RNA polymerase are approved for marketing or clinical trial, such as favipiravir, remdesivir, molnupiravir, and AT-527. Owing to structural differentiation, the underlying mechanisms of these inhibitors are diverse, many of which are not well understood. In particular, it is important to elucidate the interactions between drugs and SARS-CoV-2 polymerase on a structural basis, as in previous studies,[3,5] to determine the drug action at different stages of the RdRp catalytic cycle. This will provide a rational justification for the development of highly efficient and broad-spectrum antiviral drugs that target viral RdRp.

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