Rimantadine: A Viral Replication Inhibitor

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Abstract

The influenza virus affects thousands of people in the United States annually. Rimantadine inhibits the influenza A M2 protein thereby inhibiting viral replication[1]. It does this by binding to four sites on the outside of the transmembrane portion (Fig. 1) through hydrogen bonds and van der Waals interactions[1]. The M2 channel has developed resistance to rimantadine through mutations that destabilize the channel, making the pore easier to open. Due to drug action and resistance, a patient was advised not to take rimantadine after recently receiving the live flu vaccine.

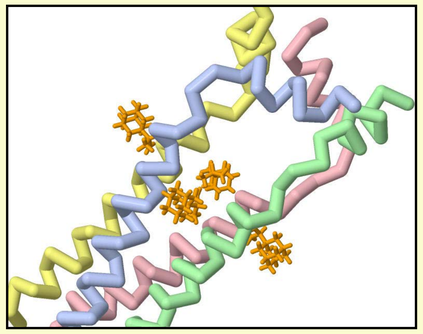

Fig. 1: Four orange rimantadine molecules bind between the four chains of the M2 channel backbone, inhibiting proton flow. Rendered from 2RLF.pdb.

Introduction

A 46 year old male presented to his pharmacy with a new prescription for rimantadine to treat influenza A. He received the live intranasal FluMist vaccination the day before. The pharmacist informed him rimantadine is not recommended since he recently received the live flu vaccination. A patient should avoid rimantadine 48 hours prior to and two weeks after vaccination with the live attenuated intranasal spray influenza vaccine as it may inactivate the vaccine[2].

Rimantadine is in the adamantane class. Influenza A contains a pH-gated proton channel in the viral envelope formed by the M2 membrane protein. The proton channel lowers the pH of the viral interior, which is required for unpacking of the viral genome and therefore for replication. Adamantane drugs inhibit movement of protons through the M2 channel, thereby inhibiting replication. Furthermore, since the 2007-2008 flu season, adamantane drugs are no longer recommended for use due to 99% resistance of influenza A (H3N2) to adamantane drugs[3]. Resistance develops through 6 amino acid substitutions to the M2 receptor[4].

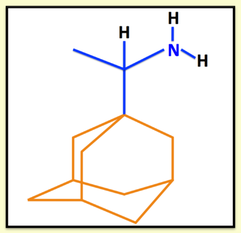

Fig. 2: Chemical structure of rimantadine. Adamantane group shown in orange. Alpha-methyl amine shown in blue.

Molecular Story

The main site of action for rimantadine is the M2 channel protein located on the viral envelope[1]. The M2 channel is a homotetrameric protein consisting of 97 residues per subunit[5]. In an acidic environment, the M2 channel becomes active and allows the passage of protons and water into the viral interior[1,5].

The M2 channel contains two important residues in its pore, His37 and Trp41. His37 serves as the pH sensor and facilitates proton conductance and Trp41 is the "gate"[1]. The Trp41 indole rings prevent the passage of water or ions via tight van der Waals interactions, which are stabilized by a nearby Asp44 residue[1]. Acidic environments protonate His37 causing conformational rearrangement and destabilization of the channel. Electrostatic repulsion from the protonated His37 residues forces the four subunits farther apart, breaking the weak van der Waals interactions between Trp41 and Asp44[1,6] thereby allowing protons and water molecules to pass through the channel and displacing water molecules that are essential for proton conduction[5] (Fig. 3).

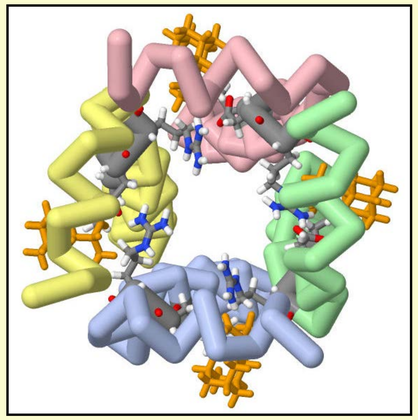

Fig. 4: Four orange rimantadine molecules binding in between the four chains of the M2 channel backbone. Rendered from 2RLF.pdb.

Rimantadine consists of an adamantane group with an alpha methylamine group[1,5](Fig. 2). Rimantadine inhibits the M2 channel by binding directly to four sites on the outside of the transmembrane portion[1] (Fig. 4). The amine head group forms hydrogen bonds with Asp44 and polar interactions with Arg45 side chains and the indole amine of Trp41[1] (Fig. 5). The adamantane group interacts with the hydrophobic walls of the channel: Ile42, Leu40, and Leu43 residues[1] (Fig. 6). Rimantadine uniquely binds to a polar patch in an otherwise hydrophobic channel[1]. This binding causes conformational changes in the channel that stabilize the closed conformation.

The M2 channel has developed resistance to rimantadine. There are several primary mutations that confer rapid resistance: V27A, A30T, and G34E within the pore and L26F, S31N, and L38F on the helix-helix interface[1] (Fig. 7,8). The mutation at residue 27 results in an enlarged pore and all other mutations destabilize helical packing, even with a bound inhibitor such as rimantadine[1] (Fig. 8). This makes the pore easier to open and disrupts the binding site of rimantadine, which is located at the interface between helices.

Knowledge of the resistance mechanism could lead to development of new, effective adamantane drugs, possibly with increased efficacy and reduced incidence of viral resistance.

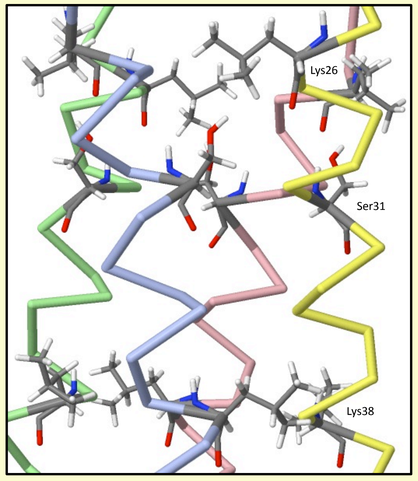

Fig. 7: Mutations at Lys26, Ser31, and Lys38 cause resistance by destabilizing helical packing. Rendered from 2RLF.pdb.

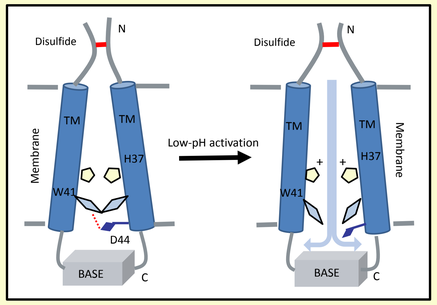

Fig. 3: Acidic pH activation of the M2 channel by protonation of His37, causing destabilization of the helix allowing for proton conduction. W=Trp, H=His, D=Asp, TM=transmembrane

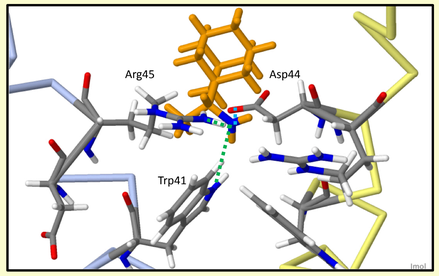

Fig. 5: Hydrogen bond interaction between the oxygen of Asp44 of the M2 channel and the nitrogen of rimantadine. Polar interactions between the rimantadine amine and Arg45 and Trp41. Rendered from 2RLF.pdb.

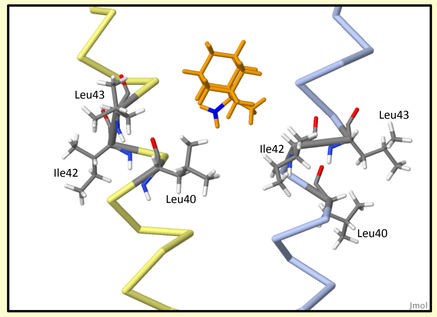

Fig. 6: Van der Waals interactions between one rimantadine molecule (orange) and Leu40, Ile42, and Leu43 on the chains of the M2 channel. Rendered from 2RLF.pdb.

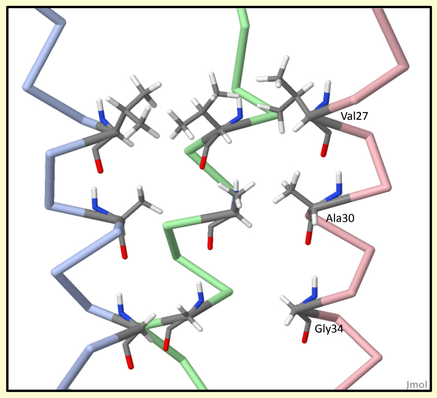

Fig. 8: Mutations at Val27 enlarge the pore. Mutations of Ala30 and Gly34 destabilize helical packing, leading to resistance. One subunit not shown. Rendered from 2RLF.pdb.

Further Drug Design

Resistance to rimantadine limits its clinical utility. There are several primary mutations of the M2 channel that confer drug resistance: L26F, V27A, A30T, S31N, G34E, and L38F6. New adamantane drugs must be developed to overcome the resistance problem. Based on drug binding, it is clear that the adamantane group and positively charged amine are critical for binding. However, the positive amine group could also be located directly off the adamantane group as in amantadine. The primary change that medicinal chemists must consider is the addition of functional groups to the positive amine to enhance binding to the M2 channel. Compounds featuring a CH_2-heteroaryl group conjugated to the amine of amantadine show promise at inhibiting the function of the S31N mutant (Fig. 9). Specifically, the heteroaryl groups were isoxazole, 1,2,4-oxadiazole, and isoxazoline[7].

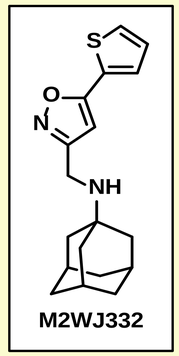

Fig. 9: M2WJ332 is comprised of amantadine conjugated to isoxazole. It inhibits the M2 mutant S31N. From Wang, et al. 2013[7].

Summary

Rimantadine inhibits the influenza A M2 channel by binding on the outside of the transmembrane portion. Through hydrogen bonding, van der Waals interactions, and hydrophobic interactions it stabilizes the closed conformation. However, the influenza A virus has developed resistance to rimantadine through six primary mutations to the M2 channel. In the development of new drugs, large heteroaryl groups can be added to the core structure of rimantadine to overcome resistance.

References

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