Analysis of the ATP Hydrolysis Rate of Hepatitis C Viral Helicase in the Presence of PNR-379

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Hepatitis C is a liver disease caused by the Hepatitis C virus (HCV) which infects over 200 million people worldwide. The HCV produces a nonstructural protein (NS3) that acts as a helicase and protease. Helicases are enzymes that hydrolyze adenosine triphosphate (ATP) and utilize the energy to move along single-stranded nucleic acids and unwind double-stranded nucleic acids. The objective of the project was to investigate the effect of an inhibitor (PNR-379) on the ATP hydrolysis rate of NS3 helicase. Since, NS3 helicase is one of the essential enzymes for processing HCV proteins and replicating the HCV, the inhibition of helicase activity can be an important strategy for treating HCV infections. ATPase activity of NS3 helicase was determined by administering a coupled spectrophotometric assay (CSA) using a Nano drop spectrophotometer. Kaleidagraph software was used to plot the rate of ATP hydrolysis versus the concentration of Poly U. In the presence of increasing concentrations of Poly U (0 to 200 μM nucleotides), the ATP hydrolysis rate of NS3 helicase was found to increase, and the maximum rate (Vmax) was ~50 s-1. In the presence of 5 μM PNR-379, the Vmax was ~48 s-1. In the presence of 25 μM PNR-379, the Vmax was ~30 s-1. The ATP hydrolysis rate of NS3 helicase was found to be reduced in the presence of PNR-379.