Original Article

A Predictive Model for Identifying the Most Effective Anti-CCR5 Monoclonal Antibody

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Abstract

CCR5 (R5) inhibition is increasingly being studied for its potential to prevent, treat, and cure illnesses. R5 is a transmembrane protein that interacts with the CD4 receptor and CXCR4 (X4) of T cells, allowing the attachment of HIV viruses to lymphocytes. Consequently, because R5 inhibition has performed well as a medicinal drug, such as maraviroc, many researchers have speculated that R5 inhibition via binding antibodies may effectively treat HIV patients. However, currently, there is a lack of information about the structural interaction between monoclonal antibodies (mAbs) and R5. The understanding of the structural CCR5 blockade via mAbs is limited. As a consequence, in this study, a predictive model with a sample size of N=160 was performed using non-linear regressions, in which the predicted reaction rates of the target R5 to gp120 interaction based on Michaelis-Menten enzyme kinetics of the inhibitor types (no, inhibitor (Control), competitive (CI), non-competitive (NI), and uncompetitive (UI)) were analyzed for their level to reduce the Vmax and Km of the R5-to-gp120 interaction.At a significant p-value of $P<0.05$, this study predicted that a non-competitive anti-R5 mAb would be the most effective inhibitor isotype since NI lowered the R5E Vmax to 20 µM/min with only a gp120S Km of 5 nM. A non-competitive anti-R5 mAb may more effectively inhibit the activity of CCR5, which may inform the production of more anti-R5 mAbs that are allosteric inhibitors of CCR5.

Keywords: Co-receptors, Monoclonal antibodies, CCR5, HIV, Glycoproteins

Introduction

Resistance to HIV-1 infection is possible when there is a mutation of a 32-base pair deletion (Δ32) in the CCR5 (R5) protein and when there is an absence of CCR5 on the cell surface [1-3]. Heterozygotes for the (Δ32) mutation also have delayed the development of the HIV illness with less rapid reductions in CD4 cell counts and reduced average viral loads [1-3]. Consequently, CCR5 is an attractive target for pharmaceutical therapies to combat HIV-1 infection [1]. When it was found that HIV enters cells by binding to the CD4 receptor [1-3], research studies focused more on forming inhibitors that could inhibit this initial binding stage of entry [1]. However, it was later discovered that the CD4 receptor was not the complete piece for the binding required for HIV entry into the host cell. It was found that a coreceptor was needed for HIV entry as well. The coreceptors CC chemokine receptor 5 (CCR5) [1] and CXC chemokine receptor 4 (CXCR4) [1-3] were revealed a few years after the discovery of CD4. CCR5 is a G protein-coupled receptor with seven transmembrane domains, in which the gp120 envelope protein from HIV binds to the N-terminus and to the extracellular loops of CCR5 to gain entry into a T cell **(Figure 1a)**.

The innate ligands of CCR5 include Regulated upon Activation, Normal T-cell Expressed, and Secreted [RANTES], macrophage inflammatory protein-1 alpha [MIP-1α], and macrophage inflammatory protein-1 beta [MIP-1β]) and these CCR5 ligands were found to be strong inhibitors of HIV entry [2, 3]. This initiated research studies to find synthetic compounds to inhibit CCR5 and block HIV entry into host cells [1]. CCR5 has surfaced on many cells including T lymphocytes, dendritic cells, and macrophages [4], in which CCR5-tropic HIV-1 strains cause the transmission of the virus [5]. Multiple products to target CCR5 inhibitors have been formed. There are multiple CCR5 antagonists, including maraviroc, aplaviroc, vicriviroc, INCB009471, and TBR 652, which have been developed. This first class of anti-HIV drugs emphasizes the host cellular pathways and notthe inhibition of viral enzyme processes [1]. Other CCR5 inhibitors in development are antibodies called PRO 140 and HGS004. PRO 140 is a humanized monoclonal

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How to cite this article: Hillman T. A Predictive Model for Identifying the Most Effective Anti-CCR5 Monoclonal Antibody. Arch Pharm Pract. 2023;14(1):40-9. https://doi.org/10.51847/d9m2zUfqr4

antibody that can bind to CCR5 and block CCR5-tropic HIV-1 viral entry [6]. HGS004 is a human immunoglobulin G4 monoclonal antibody that is a CCR5 inhibitor [1].

Monoclonal antibodies (mAbs) that act as antagonists of CCR5 have been increasingly examined [7] and have led to many potential therapeutic solutions [3, 8-20]. However, currently, there is information lacking about the structural interaction between mAbs and CCR5 [21]. Thus, the understanding of the structural CCR5 blockade via mAbs is lacking and limited. Additionally, there is a need to improve the molecular aspects of the maraviroc drug [22]. However, there is a dualism between small chemical compounds and protein-based CCR5 antagonists, in which an interaction between each may advance the improvement of the drug design for both classes of molecular CCR5 antagonist types [23]. Anti-CCR5 mAbs isotypes can target epitopes located on the amino-terminal domain, the second extracellular loops (ECL2), and other multiple extracellular domains, including the first extracellular loop (ECL1) of CCR5 [24] **(Figures 1b and 1d)**. These anti-R5 mAbs isotypes that bind to the N-terminus and the extracellular loops of R5 would directly block the binding of HIV viruses to CCR5 as a competitive inhibitor **(Figures 1b and 1d)**. An anti-R5 mAb that binds to the transmembrane domains of CCR5 is not as well-known, but this anti-R5 mAb would act more as a non-competitive inhibitor type that is allosteric **(Figure 1c)**. An anti-R5 mAb that binds to the transmembrane domains of CCR5 would be a non-competitive inhibitor because these anti-R5 mAbs do not bind to the epitopes of CCR5 that HIV-1 envelope proteins directly bind to for host cell entry **(Figure 1c)**. Another type of CCR5 inhibition could include an uncompetitive type of inhibition, in which an anti-R5 mAb binds both to an extracellular loop and to the gp120 HIV-1 envelope protein by mimicking the CD4 receptor **(Figure 1d).**

For these reasons, the present study attempts to address the research question of which molecular and structural isotype of an anti-CCR5 mAb would be the most potent for inhibiting HIV envelope protein binding to lymphocytes. Thus, this study produced a predictive model, utilizing Michaelis-Menten enzyme kinetics, to assess which type of anti-R5 mAb inhibition, either competitive, non-competitive, or uncompetitive, would be the most effective form of anti-R5 mAb inhibition. In this study, the reaction rates of the CCR5 to HIV-1 gp120 relative to the types of anti-R5 inhibition (i.e. competitive, non-competitive, and uncompetitive) were predicted by forming a non-linear regression analysis of enzyme kinetic data extrapolated from the known molarities of CCR5 as the enzyme (R5E), HIV-1gp120 envelope proteins as the substrate (gp120S), from the concentrations of anti-R5 mAb, and existing reaction rate constants specific for the R5-to-gp120 binding interactions.

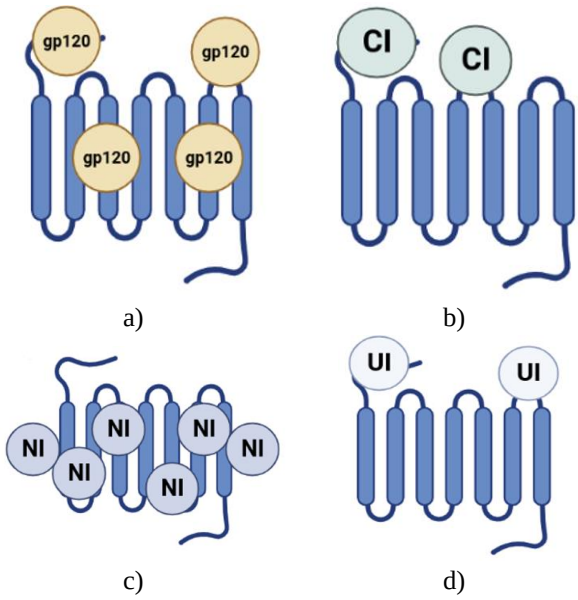


Figure 1. The Potential Binding Epitopes of anti-R5 mAbs. The figure shows the binding epitopes, in which anti-R5 monoclonal antibodies can bind. a) The binding of HIV viral envelope gp120 proteins. b) Shows the binding of the anti-R5 mAb as a competitive inhibitor. c) The non-competitive mAb binds to the CCR5 transmembrane domains. d) Displays the binding of the uncompetitive inhibitors.

Materials and Methods

Experimental Design

This study included a sample size of 160 observations (N=160), which were processed as inputs into two data sets. The study applied four conditions to the extrapolated data, which included controls (without R5 inhibitors) with three additional R5 inhibitory conditions (Competitive, Non-competitive, and Uncompetitive). A data set contained the molarities of the substrates, such as the gp120 (gp120S), and the second data set contained the velocities (uM/min-1) of the R5 (R5E), acting as the enzymatic active site for gp120S. Michaelis-Menten Enzyme Kinetic calculations were performed to derive the inputs of each data set using the molarities of R5E, gp120S, the reaction rate constants, the Km, the Ki,and the Vmax for each reaction.

Data Extraction and Extrapolation

The known protein sizes of R5 and 120, which are 40.6 kDa and 120 KDa, respectively, were used for deriving their molarities. The R5 protein acted as the enzyme, and gp120 was used as the substrate for performing the Michaelis-Menten Enzyme Kinetic analysis. The R5E and gp120S molarities were estimated according to the number of R5 molecules required for 1 T cell infection with HIV, which is 10,000 molecules of R5. The molarities of R5 (R5E) and gp120 (gp120S) were approximated close to a range of 1B to 1T molecules per T cell, which is indicative of a high viral load. Four conditions were applied to each set of data extrapolated including, without (control) an R5 inhibitor (I),

with a competitive inhibitor (CI), with an allosteric non-competitive inhibitor (NI), and with an uncompetitive inhibitor (UI). The initial molarities of data extrapolated for R5 (E) and 120 (S) substrates in the enzymatic reactions (N=200) were 30 nM and 20 nM, respectively.

Michaelis-Menten Enzyme Kinetics

The molarities of R5E, gp120S, and of the inhibitors (CI, NI, and UI), were used to calculate the Km and Ki for each enzymatic reaction. The kcat or the constant rate of the catalytic reactions (60 minutes) with the molarities of R5E, gp120S, and the inhibitors, was used to compute the Vmax for each reaction. The Michaelis-Menten equations (1-4) were used to calculate the velocities (μM/s) for the enzymatic reactions of the four conditions of no inhibitor, competitive, non-competitive, and uncompetitive inhibitors. Michaelis-Menten graphs were generated using Excel for each of the four conditions. The concentrations of the inhibitors, for the Michaelis Menten kinetic computations, were 200 μM, 500 μM, 2000 μM, and 10,000 μM. The molarities of the gp120S substrates (nM) were plotted against the velocities (μM/min) for each predictive reaction between the R5E, 120S, and the inhibitors (CI, NI, and UI).

Statistical Analysis

Descriptive statistics and unpaired t-tests were performed, using GraphPad prism, to statistically analyze all data sets of the Michaelis-Menten enzyme kinetic computations. The p-values were calculated with a statistical significance of P<0.05. A non-linear regression model, performed using Excel, of the enzyme kinetic data, was used to predict which type of anti-R5 mAb inhibitor (CI, NI, or UI) would more effectively block the interaction between R5 and gp120.

$$V=Vmax[S\}/Km+[S\} \quad (1)$$

$$V=Vmax[S]/(Km(1+[I]/Ki) + [S]) \quad (2)$$

$$V=Vmax/(1+[I]/Ki)\ [S]/Km+[S\} \quad (3)$$

$$v=(Vmax/(1+[I]/Ki))[S]/Km(1+[I]/Ki)+[S\} \quad (4)$$

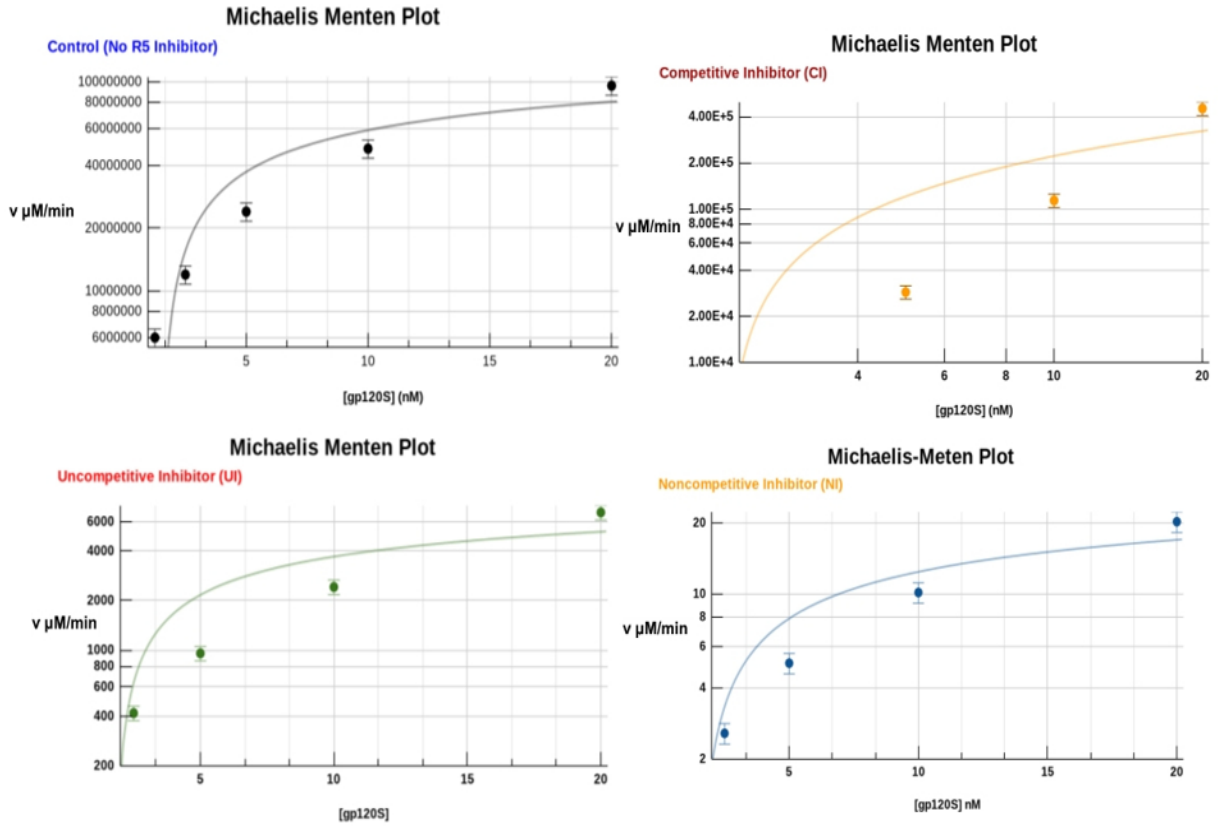
Results and Discussion

Data were extracted and extrapolated from the known molecular weights and molarities of CCR5 and the gp120 envelope protein. The reaction rate constants were extrapolated from the known rates of time for the gp120 attachment to CCR5. Using the known molarities and distributions of both R5 and gp120 on a T cell, Michaelis-Menten enzyme kinetics was performed to find the initial velocities and the predicted subsequent velocities. The CCR5 was labeled as the enzyme (R5E) and the gp120 viral envelope protein was typed as the substrate (gp120S) while the anti-R5 mAb inhibitors were classified as either competitive (CI), non-competitive (NI), or uncompetitive inhibitors (UI). The Michaelis-Menten enzyme kinetic data extrapolated was then analyzed and used as a predictive model via non-linear regressions, in which the Vmax for the control was greater than 1 billion $\mu Mmin^{-1}$. The competitive mAb inhibitor lowered the Vmax of the control to 2E+7 μmin^{-1}, and the uncompetitive inhibitor lowered the Vmax to 2 million $\mu Mmin^{-1}$. However, the non-competitive mAb inhibitor showed the most significant effectiveness as an R5 inhibitor by lowering the Vmax to 20 $\mu Mmin^{-1}$ **(Figures 2a-5a)**. The Km for the control, CI, NI, and UI were 9.5 nM, 5 nM, 7 nM, and 8 nM, respectively **(Figures 2a-5a)**.

At 200 μM of the inhibitors, the enzyme kinetic data was found to have p-values of *P*=0.047 for CI, *P*=0.046972 for NI, and *P*=0.046978 for the UI, using a one-tail T-test **(Figure 2b)**. At 500 μM of the inhibitors, the Enzyme Kinetic data was found to have p-values of *P*=0.049 for CI, *P*=0.04697 for NI, and *P*=0.04698 for the UI **(Figure 3b)**. At 2000 μM of the inhibitors, the enzyme kinetic data was found to have P-values of P=0.051 for CI, *P*=0.046970 for NI, and *P*=0.047 for the UI **(Figure 4b)**. At 10000 μM of the inhibitors, the enzyme kinetic data was found to have p-values of *P*=0.0786 for CI, *P*=0.04697 for NI, and *P*=0.0471 for the UI **(Figure 5b)**. Overall, a non-competitive mAb inhibitor of CCR5 was confirmed and forecasted by the predictive model, using non-linear regressions, to be more effective for lowering the reaction rate of R5 at a *P*<0.05, requiring a lesser potent dosage of a mAb as a non-competitive and allosteric inhibitor.

Table 1. Displayed the Vmax and Km for each Inhibitor at 200, 500, 2000, and 10,000 μM. The p-values are significant at P<0.05

Inhibitor (μM)	Vmax/Km	p-values
200		
Control	1E+8/10	
CI	3E+%/6	*P*<0.05
NI	18/5.7	
UI	5E+3/6	
500		
Control	1E+8/10	
CI	1E+6/7	*P*<0.05
NI	20/5	
UI	1E+5/10	
2000		
Control	1E+8/10	
CI	4E+6/7	*P*<0.05
NI	19/5	
UI	4E+4/6	
10000		
Control	1E+8/10	
CI	2E+7/8	
NI	19/5	*P*<0.05
UI	2E+5/9	



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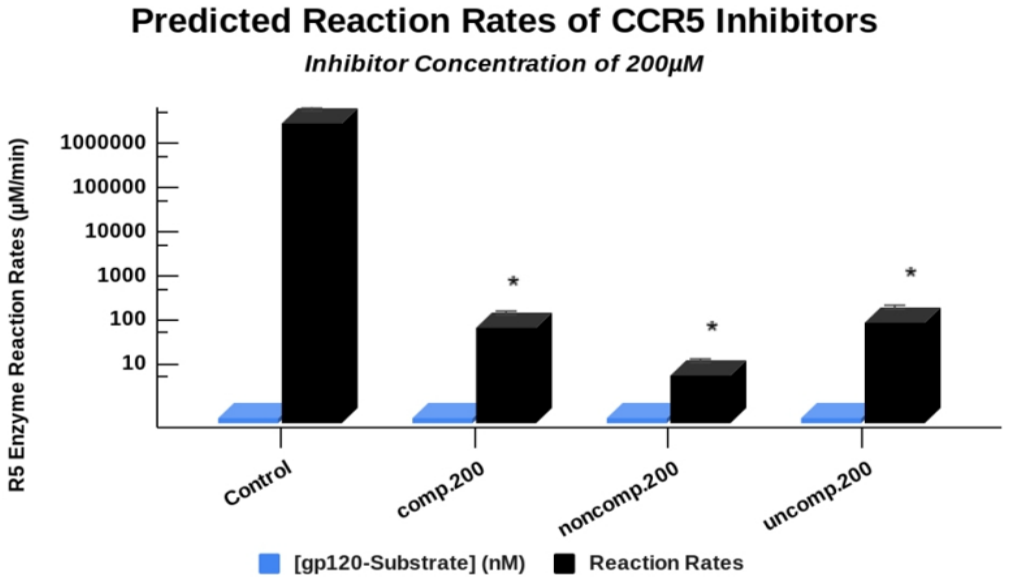
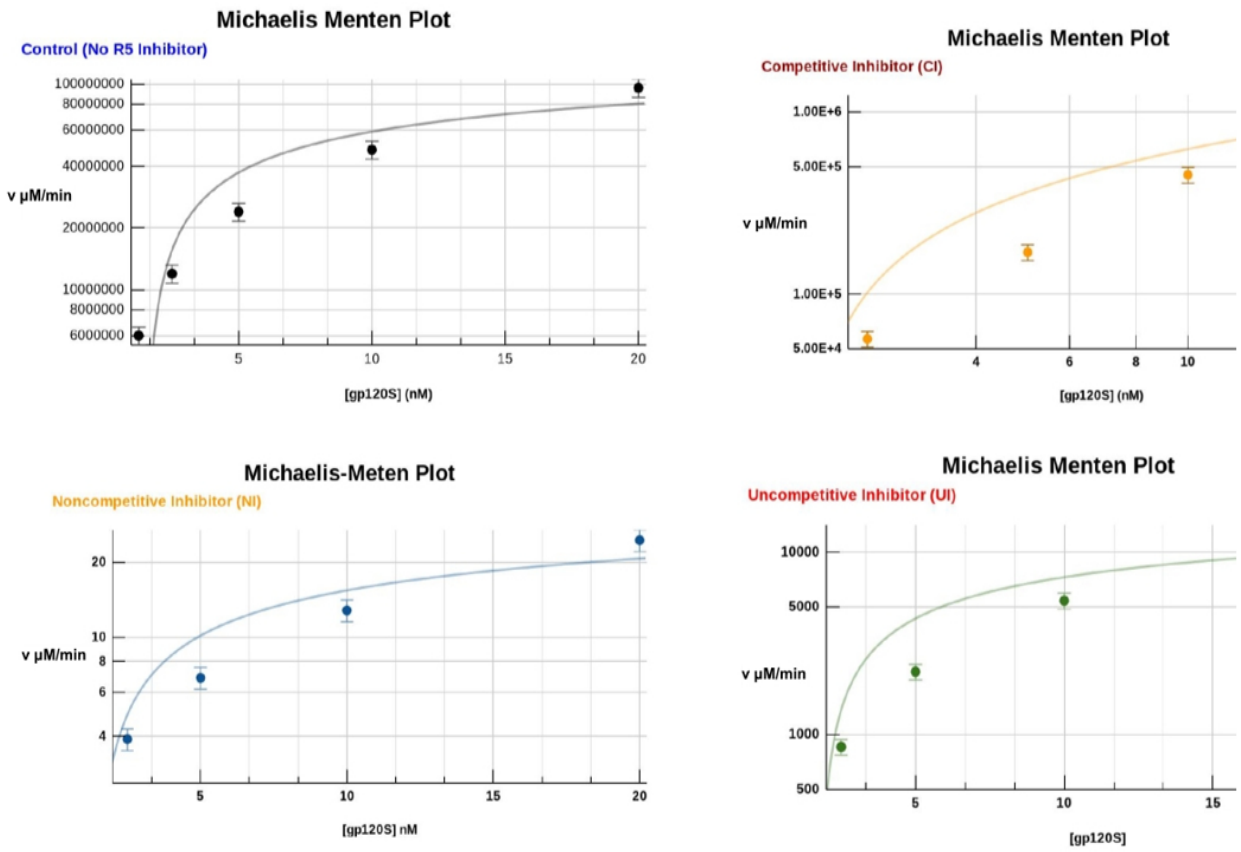


b)

Figure 2. The Michaelis-Menten Enzyme Kinetics with Non-Linear Regressions for 200 µM of inhibitors. a) The figure shows the Michaelis-Menten graphical plots for each inhibitor (Control, CI, UI, and NI), depicting the Vmax and Km for each. b) The Enzyme Kinetic data was found to have P-values of P=0.047 for CI, P=0.046972 for NI, and P=0.046978 for the UI, using a one-tail T-test. P<0.05 *



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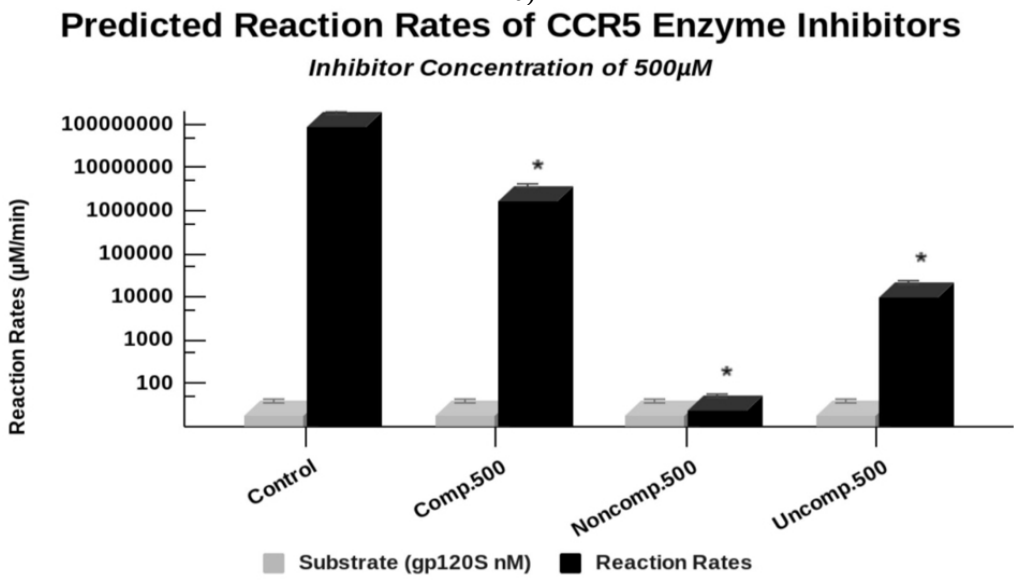
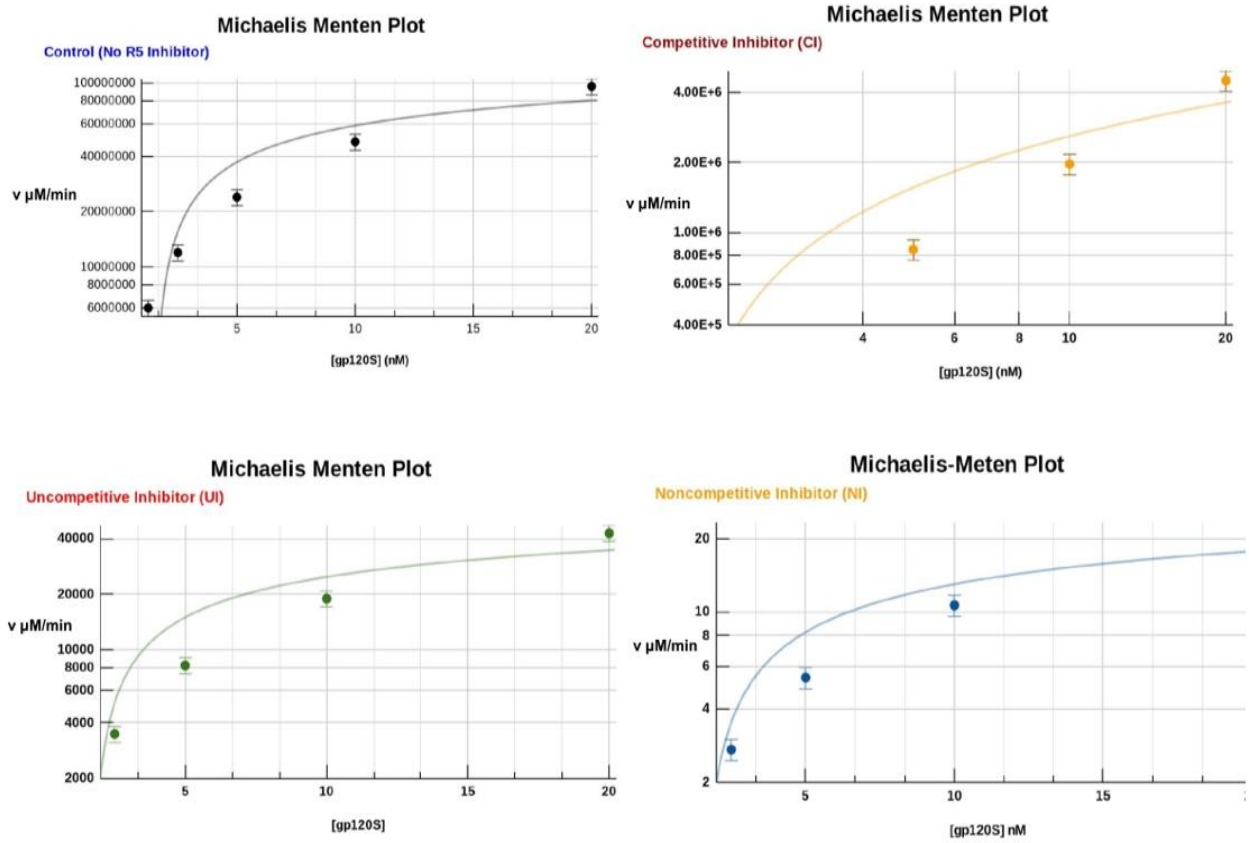


b)

Figure 3. The Michaelis-Menten Enzyme Kinetics with Non-Linear Regressions for 500 µM of inhibitor. a) The figure shows the Michaelis-Menten plots for each inhibitor (Control, CI, UI, and NI), depicting the Vmax and Km for each. b) The Enzyme Kinetic data was found to have P-values of P=0.049 for CI, P=0.04697 for NI, and P=0.04698 for the UI, using a one-tail T-test. P<0.05 *



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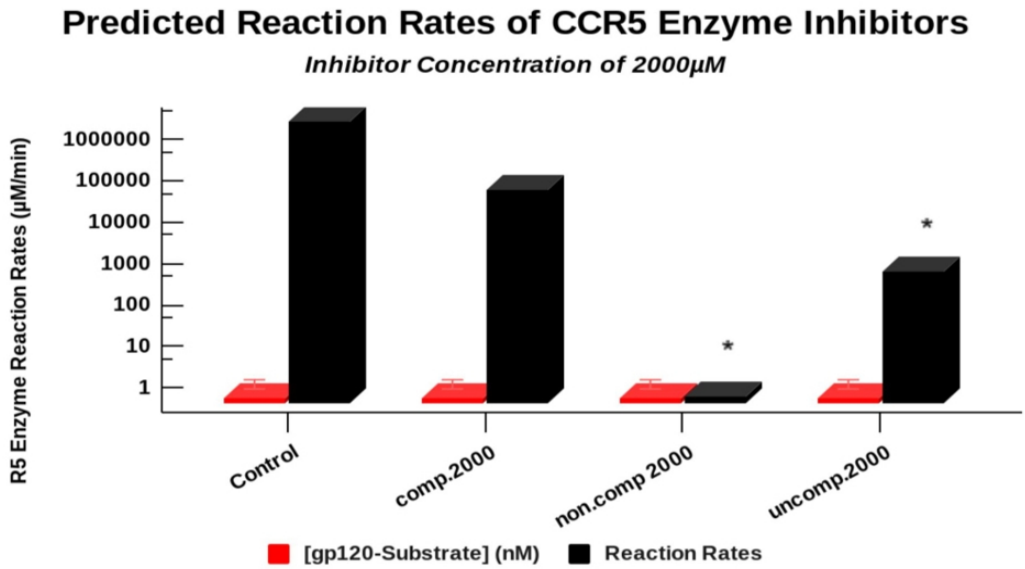
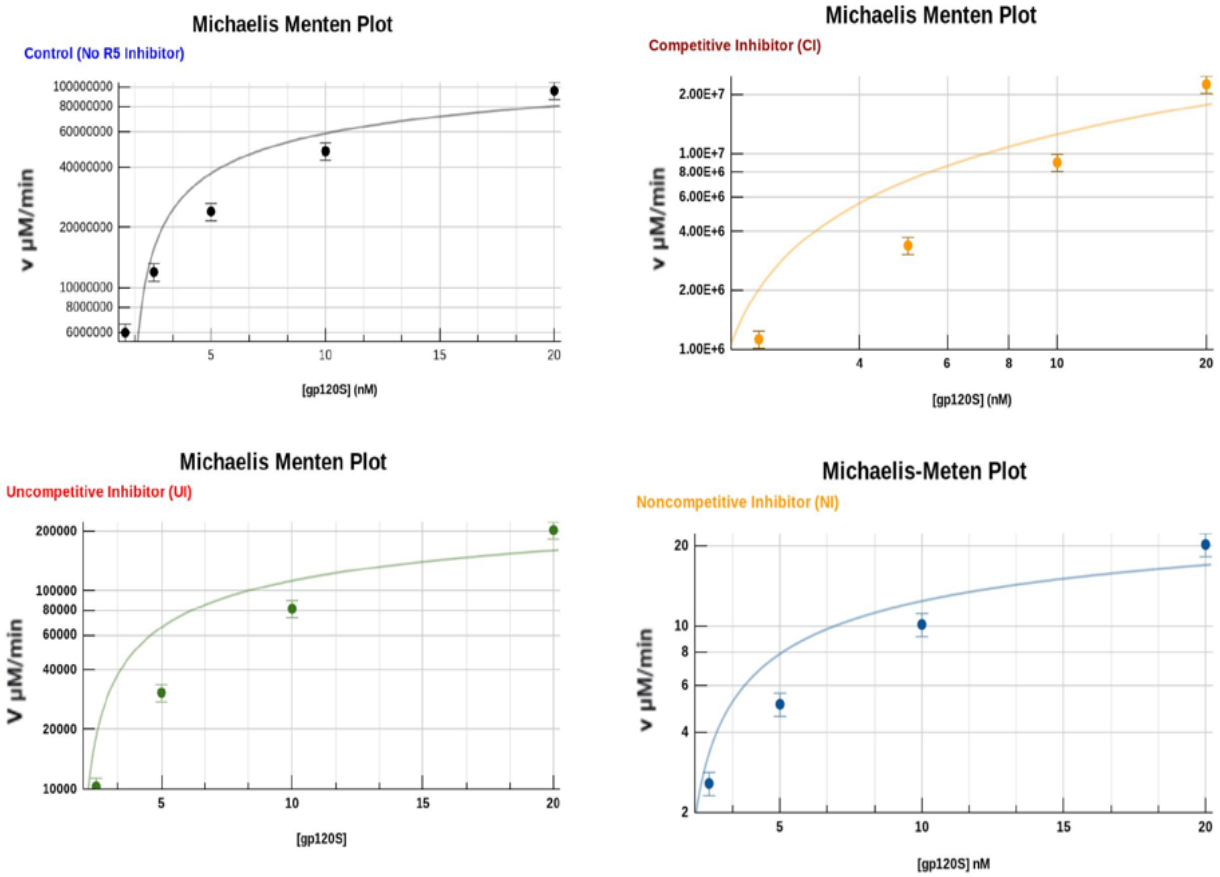


b)

Figure 4. The Michaelis-Menten Enzyme Kinetics with Non-Linear Regressions for 2000 µM of inhibitors. a) The figure shows the Michaelis-Menten plots for each inhibitor (Control, CI, UI, and NI), depicting the Vmax and Km for each. b) The Enzyme Kinetic data was found to have P-values of P=0.051 for CI, P=0.046970 for NI, and P=0.047 for the UI, using a one-tail T-test. P<0.05 *



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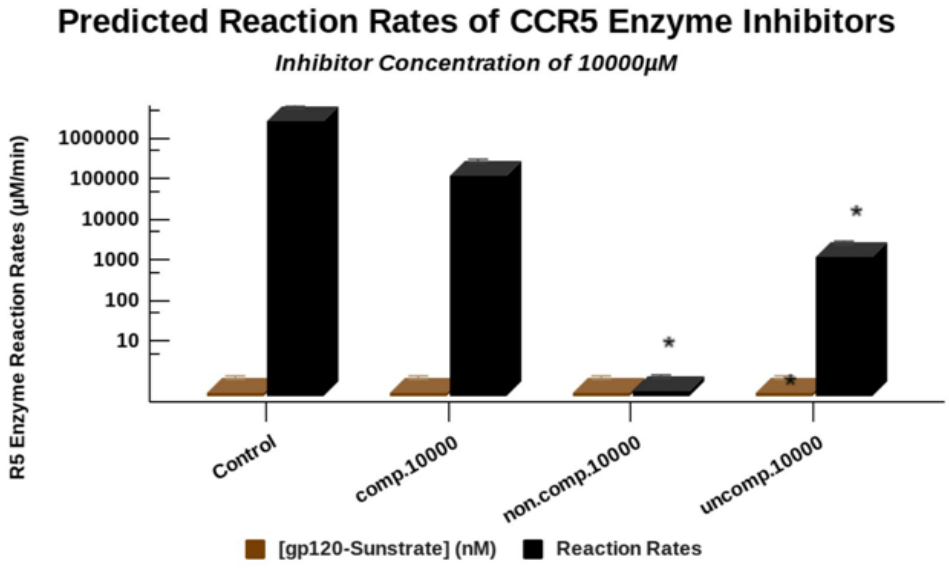

b)

Figure 5. The Michaelis-Menten Enzyme Kinetics with Non-Linear Regressions for 10,000 µM of inhibitor. a) The figure shows the Michaelis-Menten for each inhibitor (Control, CI, UI, and NI), depicting the Vmax and Km for each. b) The Enzyme Kinetic data was found to have P-values of P=0.0786 for CI, P=0.04697 for NI, and P=0.0471 for the UI, using a one-tail T-test. P<0.05 *

A CCR5 blockade via mAbs has and can potentially provide improved treatments for HIV patients. In this study, Michaelis-Menten enzyme kinetic data, of the known interaction between R5 and the HIV viral envelope protein of gp120, was used to predict the overall effectiveness of monoclonal antibody inhibitors, either competitive, non-competitive, or uncompetitive, acting against CCR5. At a significant p-value of $P<0.05$, this study identified and predicted that a non-competitive anti-R5 mAb would be the most effective inhibitor isotype since NI lowered the R5E Vmax to 20 µM/min with only a gp120S Km of 5 nM. Furthermore, antibodies that specifically bind ECL1 or the first extracellular loop do not change immune functions as shown in healthy individuals who produce natural anti-CCR5 antibodies [22] or seen in animal models, such as mice and macaques, which were stimulated to produce anti-CCR5 antibodies [23-33], using CCR5 immunization for a strategy of vaccination. Antibodies that identify the ECL2 site of CCR5 can block chemokine binding and signaling [34] versus antibodies that specifically bind to the N-terminus, in which the HIV-1 virus specifically binds to this site. For example, a mAb called 2D7 can bind to ECL2 and block HIV-1 entry into CD4 T cells, but it cannot stop the transcytosis by epithelial cells [35-37]. Using antibodies versus using chemokines or classical antiretroviral medicines can decrease complications linked to drug resistance and also the undesired functional links with other redundant CCR receptors [38].

Competitive Inhibition

A mAb anti-CCR5 inhibitor that is competitive could target the same binding site of a CCR5 antagonist known as 5P12 CCL5, which is a leading drug compound of CCL5 5M derivatives, which targets the N-terminus of CCR5 for converting CCL5 5M into an antagonist [39]. Through 3D structural modeling of CCL5 5P12 5M, the N-terminus of 5P12 differs from the N-terminus of 5P7 by an amino acid of leucine or with a threonine at position 7, respectively. Because CCR5 has a more hydrophobic region surrounding CCL5 at position 7, the leucine of 5P12 better fills this position than the threonine of 5P7 [23]. However, a possible mutation in the 5P7 CCL5-to-CCR5 complex [40] revealed possible hydrogen bonds formed by a tyrosine hydroxyl group of CCR5 S272 and one water molecule located in the crystal complex structure, which accounts for most of the observed anti-HIV-1 activity [39]. Using CCL5 derivatives as therapeutics such as for developing competitive anti-R5 mAb inhibitors, may involve the loss of tolerance because of the many inserted mutations of amino acids in the wild-type chemokines [23]. In many clinical settings, this can limit the effectiveness of CCL5-derived CCR5 antagonists due to the significant reduction of the modified chemokine to CCL5 ligand interaction [23]. Hence, anti-R5 mAb competitive inhibitors developed as CCL5 variants that target the CCR5 N-terminus can lose receptor affinity, lose tolerance, and become more easily detected by the immune system, leading to becoming a less effective CCR5 antagonist [23]. This less effectiveness of a competitive anti-R5 mAb was forecasted and confirmed by this study's predictive modeling with non-linear regressions of the enzyme inhibitor kinetics for the CI inhibitors, which produced a Vmax greater than 1E+7 µM/min, a Km of 7 nM, and lost statistical significance as the CI-inhibitor dosage increased. A current anti-R5 mAb that is a competitive type of inhibitor is the PRO140, which stops HIV from entering host cells, blocking viral replication, and does not interfere with the function of the CCR5 chemokine receptor in vitro [41-44]. PRO140 is a competitive inhibitor because it binds the extracellular sites and not the transmembrane sites of CCR5 [45].

Non-competitive Inhibition

An anti-R5 mAb antagonist that is allosteric and non-competitive can bind to the intracellular and transmembrane regions of CCR5 [23]. When an anti-R5 mAb allosteric inhibitor binds to the intracellular regions of CCR5, G protein coupling will be prevented, and its binding to the transmembrane domains would stop conformational alterations of these G proteins, including CCR5 [46-62]. Currently, allosteric CCR5 antagonists have been developed for many G proteins apart of the GPCR superfamily [23]. G protein-coupled receptors (GPCRs), such as CCR5 may be allosterically regulated by molecules binding at a site separate from the orthosteric site. Allosteric regulators are classed as negative allosteric modulators (NAM) or as positive allosteric modulators (PAM) [46]. However, there are lesser numbers of allosteric antibody modulators, but allosteric modulators still contain much promising potential as therapeutics [46].

An anti-R5 mAb that acts as a NAM for drug development holds much promise because allosteric modulators are many times more selective for their targets, and allosteric modulators can regulate the activity of endogenous ligands, which offers additional significant therapeutic benefits [46]. The increased effectiveness of antibodies as a noncompetitive inhibitor of CCR5 was predicted in this study since the NI lowered the Vmax of R5E to 20µM/min and the Km of gp120S to 5nM. Endogenous molecules, such as lipids, ions, effectors, and adaptor proteins, regulate GPCR function; however, their surfaces do not have the physicochemical properties needed for designing effective synthetic allosteric modulators [46]. Hence, anti-R5 mAbs as negative allosteric modulators may be designed to more effectively inhibit CCR5 versus synthetic modulators since mAbs are largely endogenous molecules. The drug design and development of allosteric modulators can be rapidly amplified by the present availability of crystal structures integrated with computational strategies that should assist with identifying and characterizing potential allosteric sites [2, 63, 64].

Uncompetitive Inhibition

mAbs, acting as receptor-to-co-receptor-mimetic peptides, can become potential anti-CCR5 inhibitors. Because the entry of HIV viruses requires CCR5 and CD4 on a cell's membrane, a receptor-to-co-receptor-mimetic peptide has been thought of as a potential strategy for blocking HIV entry, however, there have not been many tested in human clinical trials [41]. The UI was somewhat effective, in this study with a predicted R5E Vmax of 200,000 µM/min and a gp120S Km

of 8 nM. However, there are limitations to using antibodies as anti-R5 inhibitors. mAbs are not easily produced. mAbs are produced through bacterial, mammalian, and yeast cell lines, using hybridoma technology [7]. The production of mAbs is plagued by low yield, difficult processes of purification, and contamination. Plants have been used as alternative production sources for mAbs [7]. The findings in this study may also be limited because the differing structures, affinities, and sizes of anti-R5 monoclonal antibodies that affect antibody pharmacokinetics were not completely considered. Additionally, mAbs are more tolerated by humans than small synthetic molecules with fewer risks [7].

Conclusion

HIV is treatable and preventable with the use of antiretroviral (ARV) medications, but there are many side effects with high doses of ARVs [7]. CCR5 antibodies are potent for treating HIV. mAbs bound to CCR5 receptors could prevent HIV entry without the development of resistance, which is unlike small molecule treatments for HIV [7]. Resistance to mAbs requires further examination in the future. mAb also has lengthy elimination half-lives and will need multiple deliveries at different intervals. This would benefit HIV patients who have a continued requirement for daily therapies and would take advantage of infrequent dosing options [7]. Future research on the use of CCR5 mAbs is required that may assist patients who are resistant to all antiretroviral medicines [7]. The importance of this study includes that it identifies non-competitive mAbs for more effectively inhibiting CCR5 than competitive and uncompetitive anti-R5 mAb isotypes. This study may be significant for developing highly effective monoclonal antibodies for inhibiting CCR5 via non-competitive inhibition.

ACKNOWLEDGMENTS: A special thanks to my many mentors over the years, including Dr. Tobin. Thank you.
CONFLICT OF INTEREST: None
FINANCIAL SUPPORT: None
ETHICS STATEMENT: None

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