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Structural bioinformatics

Identification of potential HIV-1 targets of minocycline

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ABSTRACT

Summary: Minocycline, a broad spectrum antibiotic, has been discovered to have inhibitory activity against HIV-1 *in vitro*, but the targets inhibited are unknown. We used a docking with dynamics protocol developed by us to predict the binding affinities of minocycline against seven active sites of five HIV-1 proteins to putatively identify the potential target(s) of minocycline. The results indicate that minocycline has the highest predicted binding affinity against HIV-1 integrase.

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1 INTRODUCTION

Minocycline is a broad spectrum antibiotic approved by the US Food and Drug Administration for treatment of bacterial infections. Minocycline has been discovered to substantially inhibit replication of HIV-1 in primary cultures of microglia (Si *et al*., 2004), macrophages and lymphocytes (Zink *et al*., 2005). Experimental evidence has shown that the p38 mitogen-activated protein kinase cascade is critical for HIV-1 replication (Darcissac *et al*., 2000), and that blockade of this signal transduction pathway may be a primary mechanism of minocycline for suppression of HIV-1 infection (Darcissac *et al*., 2000; Zink *et al*., 2005). Previous studies have pointed to evidence that minocycline is unlikely to have classic antiviral activity against HIV-1, such as reverse transcriptase and protease inhibition, since minocycline was not engineered to target a specific viral protein (Zink *et al*., 2005). However, there is no clear evidence to support this conclusion.

2 METHODS

We hypothesized that minocycline inhibits HIV-1 proteins that are essential for viral replication. To test this hypothesis, we used a computational docking with molecular dynamics (MD) simulation method developed by us (Jenwitheesuk and Samudrala, 2005) to dock minocycline into seven active sites of five HIV-1 proteins to identify the putative targets of minocycline. We downloaded the X-ray diffraction structures of HIV-1 capsid, gp41 HR1 (theoretical structure), integrase, protease, and reverse transcriptase from the Protein Data Bank (PDB). For each structure, the inhibitor was first removed from the active site and mutant side chains in these structures were then substituted to the wild-type side chain using SCWRL version 3.0. Protein structures from this step were used as a template for protein–inhibitor binding energy and cutoff calculations.

Protein–inhibitor docking calculations were carried out using AutoDock version 3.0.5 with a Lamarckian genetic algorithm as described in (Goodsell *et al*., 1996). We performed preliminary docking experiments to locate the potential binding sites of minocycline by generating a grid box that is large enough to cover the entire surface of each protein active site, as described by the experimentalists who solved the structures. The protein–minocycline complex derived from the preliminary docking procedure was consequently allowed to relax using MD simulation. The MD simulations are carried out using the X-PLOR software version 3.851. The topology and parameters for all inhibitors were obtained from the PRODRG server (Schuttelkopf and van Aalten, 2004). One hundred steps of energy minimization of the protein–inhibitor complex were initially performed, followed by MD simulation at 300 K. The trajectories at 0.1 ps were recorded and processed in a second docking step using similar docking parameters as used in the preliminary docking procedure.

We determined the binding affinity between a protein and its inhibitor by calculating the inhibitory constant (K_i) of the protein–inhibitor complex, which is correlated to the concentration at which 50% of the protein is inhibited (IC50). AutoDock generates three binding energy terms: intermolecular energy, internal energy of the ligand, and torsional free energy. The final docked energy was calculated from the sum of the intermolecular energy and the internal energy of the ligand. The free energy of binding was calculated from the sum of the intermolecular and the torsional free energies, and consequently converted into a K_i according to Hess's law. The lowest-energy solution was accepted as the calculated binding energy and its K_i value was used to define the binding affinity of the inhibitors. Further details of the MD simulation and docking protocols are given elsewhere (Jenwitheesuk and Samudrala, 2005).

To predict the inhibitory constant cutoff, we downloaded the X-ray diffraction structural complexes of 89 HIV-1 inhibitors (1 integrase inhibitor, 32 non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 56 protease inhibitors) from the PDB. For each wild-type structure generated in the first step, we docked its inhibitor into the active site and predicted its K_i value. The mean predicted K_i of each group of HIV-1 proteins was used as the cutoff to identify potential target(s) of minocycline. Although there were several nucleoside reverse transcriptase inhibitors (NRTIs) available in the PDB, we did not predict the cutoff value for this target due to its different inhibitory mechanism; i.e. NRTI is a chain terminator of the newly forming viral DNA, whereas minocycline tends to occupy the NRTI active site and does not directly interact with the viral DNA. Therefore, their predicted K_i values are not comparable.

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Table 1. Predicted inhibitory constants (K_i) of 89 HIV-1 inhibitors and minocycline against wild-type structures of five HIV-1 proteins

HIV-1 target	PDB identifier	Number of inhibitors	Calculated inhibitory constant (K_i)	
			Cutoff	Minocycline (rank)
Capsid C-terminal	1E6J	0	–	1.23×10^{-4}
Capsid N-terminal	1E6J	0	–	7.38×10^{-5}
gp41 HR1 groove	1IF3	0	–	Not bind
Integrase	1BIS	1	3.18×10^{-5}	2.29×10^{-7} (1)
Protease	1GNO	56	7.76×10^{-6}	3.74×10^{-6} (38)
Reverse transcriptase (NRTI-binding site)	1IKW	0	–	1.28×10^{-5}
Reverse transcriptase (NNRTI-binding site)	1IKW	32	6.35×10^{-6}	1.06×10^{-4} (33)

The lower the predicted K_i value, the greater the predicted binding affinity. Since the size of the inhibitor set was small and the range of the calculated K_i s were large, the cutoff value for each protein was calculated from mean predicted K_i of the inhibitors of that protein, and was used to identify the potential target(s) of minocycline. The result shows that minocycline had no affinity with the HR1 groove of gp41, and bound to C-terminal and N-terminal of capsid, and the active sites of NRTI and NNRTI with low affinity. In contrast, minocycline had moderately high binding affinities against integrase and protease with calculated K_is of 2.29×10^{-7} and 3.74×10^{-6}, respectively.

3 RESULTS

We previously showed that our docking with dynamics protocol, that integrates protein flexibility using molecular dynamics simulations with an inhibitor flexible-docking technique, has higher accuracy in predicting binding energies of HIV-1 protease–inhibitor complexes than that of the original inhibitor flexible-docking protocol. The calculated binding energies and the calculated K_i predicted by docking with dynamics were highly correlated with the experimental binding energies and the IC50, with correlations ranging from 0.35 when no protein dynamics was incorporated to 0.88 when dynamics was incorporated for a set of 25 HIV-1 protease–inhibitor complexes (Jenwitheesuk and Samudrala, 2003; Jenwitheesuk and Samudrala, 2005). This higher correlation enables us to apply this technique to identify the potential HIV-1 targets of minocycline.

In this study, our docking results indicate that minocycline has moderately high binding affinity against HIV-1 integrase and protease with a predicted K_i of 2.29×10^{-7} and 3.74×10^{-6}, respectively (Table 1). Minocycline was ranked 38th among 56 protease inhibitors and its predicted K_i fell within 0.5 SD above the cutoff (of 7.76×10^{-6}). In contrast, minocycline had no affinity with the HR1 groove of gp41, and bound to the other four active sites (C-terminal and N-terminal of capsid, and the active sites of NRTI and NNRTI) with low affinity, concurring with previous experimental studies that minocycline had low inhibitory activity against HIV-1 reverse transcriptase (IC50 = 1200 μM) (Wondrak *et al.*, 1988).

We then inspected the binding modes of minocycline on the active sites of integrase and protease to determine the most likely target. Minocycline has four connecting hexagonal rings making its structure too rigid and too short to fit in the four subpockets of protease. Therefore, it is less likely that minocycline would have high inhibitory activity against HIV-1 protease, though the structural flexibility and the molecular size of minocycline may be increased by dimer formation. Minocycline, on the other hand, has similar shape and size to that of 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone (5ClTEP), an inhibitor designed to target HIV-1 integrase (Goldgur *et al.*, 1999). We predicted that minocycline binds to the integrase active site with high binding affinity, and interacts with the Mg^{2+} ion in a similar manner to 5ClTEP (Fig. 1).

Previous docking studies on target identification of curcumin against HIV-1 integrase and protease using the same docking software showed a high correlation between the predicted binding energies and the experimental IC50s, indicating the accuracy of the software in predicting protein–inhibitor binding energies (Vajragupta *et al.*, 2005). Curcumin was predicted to have high binding affinity against HIV-1 integrase (predicted binding energy was −8.79 kcal/mol) which was comparable to that of 5ClTEP against HIV-1 integrase (−8.25 kcal/mol). In contrast, the predicted binding energy of curcumin against protease (−9.77 kcal/mol) was much weaker than that of L745,524, a known protease inhibitor (−18.40 kcal/mol). By using the predicted binding energies of HIV-1 inhibitors as a reference, curcumin was predicted to target HIV-1 integrase. The experimental (IC50) results indicate that curcumin had an IC50 of 30–40 μM against HIV-1 integrase (Artico *et al.*, 1998), which was more potent than that of curcumin against HIV-1 protease (100 μM) (Sui *et al.*, 1993).

In the context of these experimental studies, our own study then suggests that minocycline is likely to bind and display inhibitory activity against HIV-1 integrase. We propose that, in addition to the inhibitory mechanism on p38 mitogen-activated protein kinase cascade, minocycline may suppress HIV-1 infection by inhibiting HIV-1 integrase. However, there are several other HIV-1 proteins whose 3D structures were not included in this study and they might be minocycline targets as well. Finally, there is also the consideration that minocycline indirectly inhibits replication by interfering with host mechanisms that HIV relies on, for example by reducing concentrations of inflammatory cytokines that facilitate HIV-1 replication.

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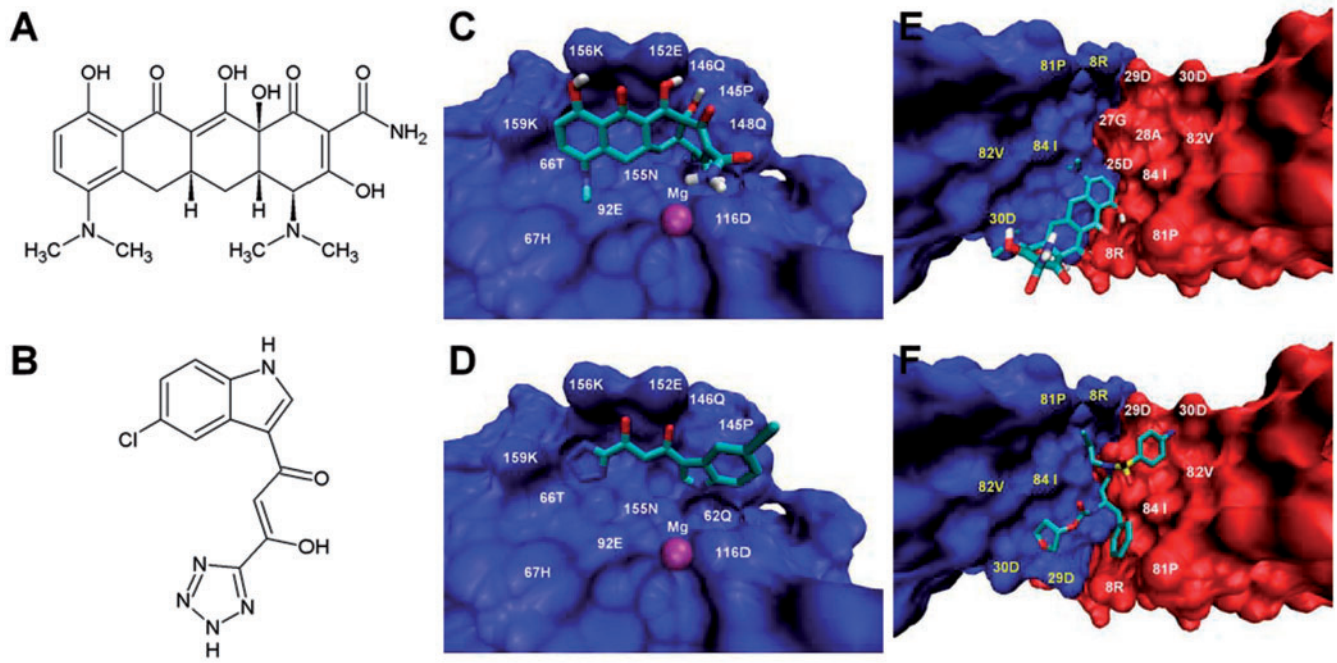

Fig. 1. Structural comparison of minocycline (**A**), a broad spectrum antibiotic, and 5ClTEP (**B**), an HIV-1 integrase inhibitor. Minocycline (**C**) has a similar shape and size, binds to the integrase active site with high binding affinity, and interacts with the Mg^{2+} ion, in a similar manner to 5ClTEP (**D**). Minocycline has four connecting hexagonal rings making its structure too rigid and too short to fit in the four subpockets of HIV-1 protease (**E**). In contrast, amprenavir, which was designed to specifically inhibit HIV-1 protease, has four subgroups that fit well with the four subpockets of the protease (**F**). Therefore, it is less likely that minocycline would have high inhibitory activity against HIV-1 protease, though the structural flexibility and the molecular size of minocycline may be increased by dimer formation causing it to have protease inhibitory activity. The similarity of the binding modes of minocycline and 5ClTEP suggests that HIV integrase may be one of its potential targets.

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Conflict of Interest: none declared.

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