Probing the chemical interaction space governed by some NSAIDs and *Pseudomonas aeruginosa* Elastase B

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Abstract

Due to its elastolytic activity, *Pseudomonas aeruginosa*, a very well-known opportunist gram-negative bacteria, can cause severe tissue damages and tissue hemorrhages. Therefore, blocking its extracellular proteases, such as elastase B can be used as a strategy to confront *P. aeruginosa*. Non-steroidal anti-inflammatory drugs, also known as NSAIDs, are among the most popular drugs used against microbial infections. Herein, chemical interaction spaces of famous NSAIDs named Ketoprofen, Naproxen, and Ibuprofen have been investigated against bacterial elastase as well as human elastase to determine the affinity and selectivity of these drugs for their receptors. Optimized structures of ligands and receptors were subjected to molecular docking simulations, applying AutoDock Vina plugin available in PyRx software. Docking results as well as non-covalent interaction space analyses revealed suitable binding energies for all NSAIDs/receptor complexes. However, better docking scores as well as richer chemical interaction spaces were observed in case of NSAIDs/bacterial receptor complexes. This can suggest higher affinity and better selectivity of these drugs against bacterial elastase.

Key words: *Pseudomonas aeruginosa*; Elastase; NSAIDs; docking simulation

Introduction

Bacterial infection can lead to severe and life-threatening health issues, and is a significant threat to simple injuries. In case of burns, treating infections caused by *Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogenic bacterium, is the main therapeutic challenge which sometimes can fail and lead to patient death. Since *P. aeruginosa* is highly capable of developing resistance to antimicrobials, infections caused by *P. aeruginosa* are very hard to treat. Therefore, sustained and efficient antibacterial treatments must be considered against *P. aeruginosa* infections (1). Bacterial proteolytic activity is a major contributor to infection development. *P. aeruginosa* proteases contribute to defense against immune responses of hosts as well as serum bactericidal activity (2, 3). Alkaline protease, elastase A, and elastase B are the main proteases produced by *P. aeruginosa* strains. Elastolytic activity is a major virulence factor during the acute phase of *P. aeruginosa* infection, while the role of alkaline protease in bacterial invasion is less important (2). Elastase B (LasB) is a zinc-dependent metalloprotease, responsible for lung hemorrhages and corneal tissue destruction (Fig. 1). Additionally, LasB can cleaves host proteins including elastin, collagen, and fibrin. Similar to antibiotics, Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed as safe anti-inflammatory agents in infection chemotherapy (4, 5). Antibacterial potential of NSAIDs against *P. aeruginosa* has been previously investigated (4, 5). To shed light on the chemical interaction spaces governed by bacterial LasB and NSAIDs, herein, an in silico investigation was conducted to analyze binding potential and interactions of three Non-steroidal anti-inflammatory drugs (Ketoprofen, Naproxen, and Ibuprofen) against elastase B of *P. aeruginosa*. To further investigate the selectivity of the NSAIDs against bacterial elastase, the same analysis has been performed for human elastase.

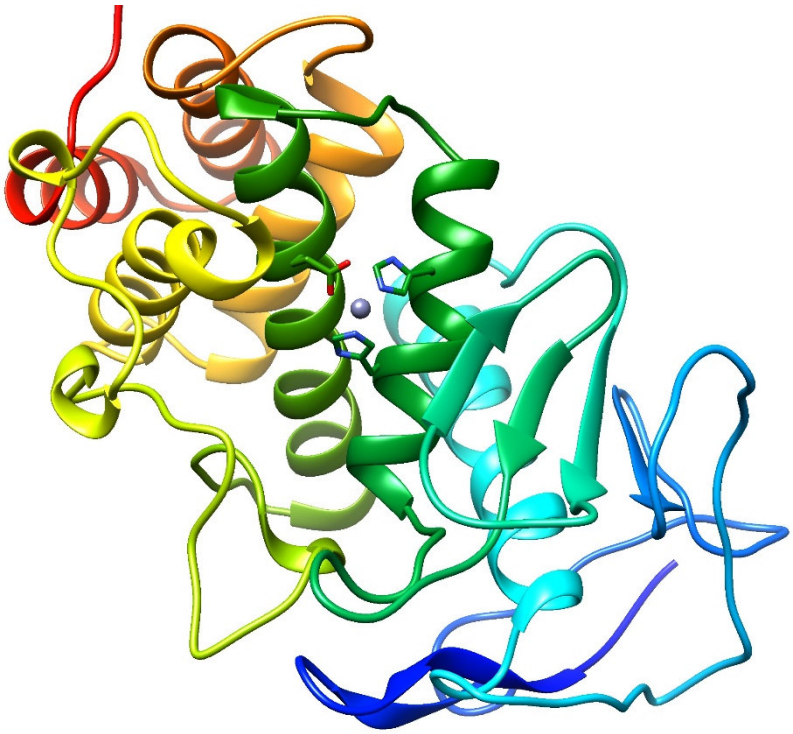

Fig. 1: 3-D structure of elastase (PDB ID: 3dbk)

Methods

Preparation of chemical structures

Elastase receptors

The PDB structures of bacterial (PDB ID: 3DBK) and human elastases (PDB ID: 3Q77) were obtained from Protein Data Bank (PDB). Co-crystallized ligands and water molecules were removed from PDB structures prior to molecular docking simulations. Afterwards, polar hydrogens were added, and partial atomic charges were assigned applying the Gasteiger method (6). Energy minimization was then performed utilizing the 1000 steepest descent gradient algorithm (7, 8).

NSAIDs

The Structured Data Files (SDFs) of Ibuprofen, Naproxen, and Ketoprofen were obtained from the

PubChem chemical library (9). Structures were then geometrically optimized in SYBYL7.3. Optimizations were carried out applying Tripos force field with distance-dependent dielectric and Powell conjugate gradient algorithm with convergence criterion of 0.01 kcal/mol Å. Gasteiger method was used to calculate the Partial atomic charges (6).

Docking simulation analysis

Molecular docking simulations were performed to investigate the chemical interaction space governed

by NSAIDs and elastases. Prior to docking, structures of ligands and receptors were prepared as stated before. Afterwards, the SDF fies of Ibuprofen, Naproxen, and Ketoprofen were one by one imported into OpenBabel software to be converted to PDBQT format. Energy minimizations were then run via the Universal Force Field (UFF) algorithm (10). The AutoDock Vina (11) plugin integrated into the PyRx software (10) was applied to run molecular docking simulations. The simulation runs were conducted on a desktop equipped with an Intel® Core™ i7-8700K 3.60 GHz processor and 24 GB DDR4 memory. Finally, conformations with the lowest binding energy were selected and their chemical binding patterns were analyzed using Discovery Studio Visualizer and Chimera software.

Results

Based on docking simulations outputs, the best binding energy calculated for complex of bacterial elastase with Ibuprofen, Naproxen, and Ketoprofen were, respectively, -5.8 kcal/mol, -5.9 kcal/mol, and -6.7 kcal/mol (Table 1). Correspondent binding energies for human elastase/NSAIDs complexes were, respectively, -5.5 kcal/mol, -5.4 kcal/mol, and -6.1 kcal/mol (Table 1). This suggests better affinities of three investigated NSAIDs for bacterial enzyme compared to that for human enzyme.

Table 1: Docking binding energy (kcal/mol) of complexes

Receptor ID	Docking binding energy (kcal/mol)		
	Ibuprofen	Ketoprofen	Naproxen
3DBK	-5.8	-6.7	-5.9
3Q77	-5.5	-6.1	-5.4

Major non-covalent interactions governing the chemical spaces of NSAID/elastase complexes are listed in Table 2.

Table 2: number of non-covalent interactions in each complex

Interaction type	Number of interactions					
	Ibuprofen/3DBK	Ketoprofen/3DBK	Naproxen/3DBK	Ibuprofen/3Q77	Ketoprofen/3Q77	Naproxen/3Q77
Hydrogen bond	4	2	2	1	2	2
Hydrophobic interaction	3	6	7	5	3	3

Furthermore, two dimensional views of NSAID/bacterial elastase complexes and NSAID/human elastase complexes are illustrated in Figure 2 and Figure 3, respectively.

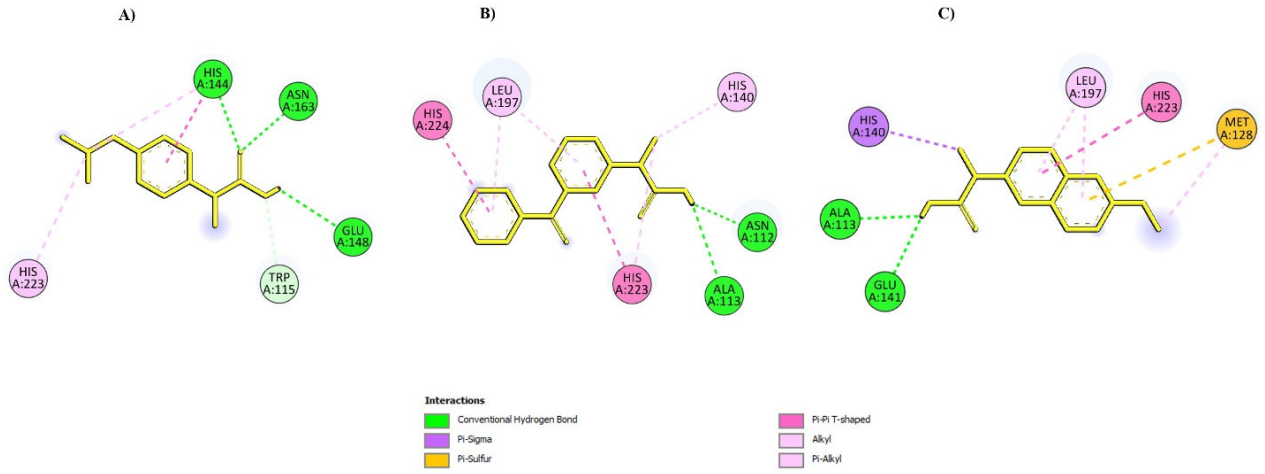

Fig. 2: Two dimensional views of NSAID/bacterial elastase complexes. A), B) and C) are, respectively, Ibuprofen, Ketoprofen and Naproxen

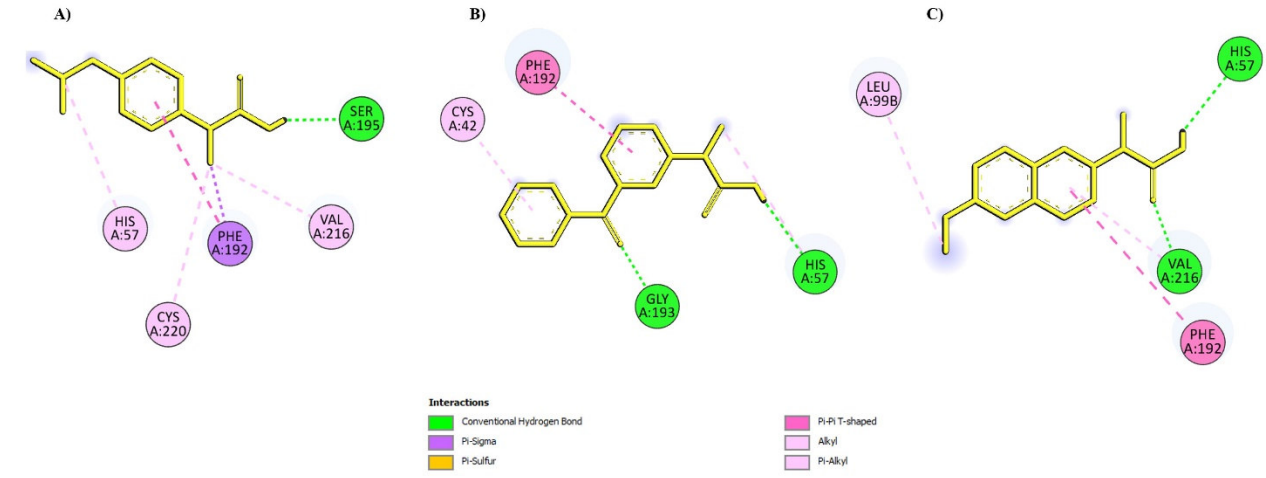

Fig. 3: Two dimensional views of NSAID/human elastase complexes. A), B) and C) are, respectively, Ibuprofen, Ketoprofen and Naproxen

Based on docking results, Ibuprofen interacts with bacterial elastase through four hydrogen bonds and three hydrophobic interactions made by Trp115, His144, Glu148, Asn163, and His223 residues (Figures 2 and 3). Human elastase, however, makes six non-covalent interactions (one hydrogen bond and five hydrophobic interactions) with Ibuprofen through His57, Phe192, Ser195, Val216, and Cys220 residues (Figures 2 and 3). In case of Ketoprofen, number of weak interactions observed for bacterial and human enzymes are, respectively, eight and five (Table 2). In bacterial enzyme, residues Asn112 and Ala113 are responsible for making hydrogen bonds, while residues His57 and Gly193 participate in making hydrogen bonds in human enzyme (Figures 2 and 3). Six hydrophobic interactions, made by Leu197 and His140,223,224, were observed within Ketoprofen/bacterial elastase complex, whereas number of hydrophobic interactions observed for Ketoprofen/human elastase was three (made by Cys42 and Phe192) (Figures 2 and 3). As reported in Table 2, there are nine non-bonded interactions between Naproxen and bacterial elastase, while five non-bonded interactions are present between Naproxen and human elastase. Residues Ala113 and Glu141 are responsible for making hydrogen bonds between Naproxen and bacterial elastase, while Met128, His 140,223 and leu127 of bacterial enzyme interact through hydrophobic interactions with Naproxen (Figures 2 and 3). In case of Naproxen/human elastase complex, however, hydrogen bonds are made by residues His57 and Val216, whereas hydrophobic interactions are made by Leu99 and Phe122 residues (Figures 2 and 3). To sum up, in case of all three NSAIDs, total number of weak interactions in NSAIDs/bacterial elastase complexes are higher than that of in NSAIDs/human elastase complexes, suggesting higher affinity of these NSAIDs for bacterial enzyme. This is also in very good agreement with docking calculated binding energies (Table 1).

Discussion

Extracellular proteases, especially elastases A and B, are major contributors to acute infections caused by *P. aeruginosa*. Elastin significantly contribute in the tissue integrity of human, including lung tissue and blood vessels. The elastolytic activity of *P. aeruginosa* plays major roles in infection expansion and tissue damages, especially in respiratory patients, leading to hemorrhages (3). Therefore, inhibiting the elastolytic activity of this bacterium is of great essence to prevent acute complications and subsequent tissue damages. Herein, an in silico study has been performed to investigate chemical interaction spaces of Ketoprofen, Naproxen, and Ibuprofen against elastase B of *P. aeruginosa*. Furthermore, in order to compare the selectivity of the NSAIDs against bacterial elastase with that for human elastase, the same analysis has been performed for human enzyme. Based on docking simulation results, in case of all three drugs, better docking scores were observed for NSAIDs/bacterial elastase complexes. Scores of -6.7, -5.9, and -5.8 were, respectively, observed for Ketoprofen, Naproxen, and Ibuprofen against bacterial elastase. Moreover, chemical interaction space analysis revealed that regarding all three investigated drugs, number of non-bonded interactions is higher when they interact with the bacterial elastase. Therefore, it can be concluded that these NSAIDs have better affinity for bacterial enzyme.

Conclusion

Pseudomonas aeruginosa is associate with many acute/chronic infectious disorders, including wound, respiratory tract, and urinary tract infections. Since elastolytic activity of *Pseudomonas aeruginosa* is highly engaged with pathogenic characteristics of this pathogen, blocking its extracellular proteases, such as elastase B can be considered as an efficient strategy to overcome pathogenicity of *P. aeruginosa*. Non-steroidal anti-inflammatory drugs such as Ketoprofen,

Naproxen, and Ibuprofen are among the most popular chemicals applied against microbial infections. Herein, *in silico* docking simulations have been carried out to investigate chemical interaction space governed by some NSAIDs (Ketoprofen, Naproxen, and Ibuprofen) and both human and bacterial elastases. Docking and non-covalent interactions analyses revealed better results for Ketoprofen, Naproxen, and Ibuprofen against bacterial elastase, suggesting that they have better affinity for bacterial enzyme.

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