Computational Analysis of Pen-110 Binding To Cd-61 of Platelets
Hammad Tufail Chaudhary

ABSTRACT

OBJECTIVE: To review the docking of PEN-110 at CD-61 which would give the idea about the effect of PEN-110 on function of platelets.

STUDY DESIGN: An observational study.

PLACE AND DURATION: At Medical College, Taif University, Taif, Saudi Arabia from March 1st, 2016 to September 30th, 2016.

METHODOLOGY: The study was carried out in-silico. PDB (Protein data bank) code of Tirofiban bound to CD-61 was 2vdm. CD-61 (2vdm) was docked with Tirofiban as a control using online docking tools i.e. Patchdock and Firedock. Then, PEN-110 and CD-61 were also docked together. Best docking poses and their interactions to active sites of 2vdm were found. Then comparison of Hydrogen Bonds, Hydrogen Bond Lengths, Hydrophobic bonds of 2vdm molecule and best poses of docking results were done.

RESULTS: The Hydrogen bonds and Hydrogen bond length and Hydrophobic bonds of docking results were compared to 2vdm. No docking to active site was observed in Patchdock. In firedock docking, PEN-110 docked to active site of CD61, but the global energy of docking was significantly less than the global energy of binding of Tirofiban with CD61 i.e. 101.9 in comparison to -5.99, respectively.

CONCLUSION: PEN-110 binds to the active site of CD-61 with weaker binding force. We can establish that PEN-110 does not affect this important receptor of platelet which is needed for proper function of platelets.

KEYWORDS – Platelets, Receptors, Computational Analysis, Pen-110 Binding, CD-61, Docking.

HOW TO CITE THIS:

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Blood transfusion’s importance cannot be denied for patient care. Therefore, lot of effort has been incorporated into making the transfusion safe. Viral screening and nucleic acid testing has been part of that in last few years ¹. In spite of all these struggle aim of safe transfusion has not been achieved ².

Growth of bacteria in platelet bags has been one of the great issues to be addressed. Room temperature storage and biological condition are key factors for the spread of bacteria in platelet bags ³. That is the reason that rate of platelet bag contamination was shown as 1/2,000-3,000 units ⁴. In one study, ratio of severe sepsis to infected transfusion was found to be 1/6 ⁵.

Pathogen reducing technology (PRT) has emerged to cope with situation of bacterial growth in blood bags. Riboflavin, the essential vitamin B2, and methylene blue, psoralens, such as S-59 (amotosalen-HCl) and S-303 or the ethylene imine PEN-110 are among the PRT ⁶⁷. PEN 110 needs not light for activation. Changes in pH lead to its activation. It is also noted that it does not affect the morphology and physiology of platelets. Furthermore, it doesn’t damage red cells. Only demerit which has been reported till now is that it produces antibody against red cells ⁸.

Clotting of blood is controlled by platelets, and CD (Cluster of differentiation)-61 is one of the important antigens on platelets. CD-61 binds to fibrinogen, which is helpful for homeostasis. This property of this receptor leads to development of several drugs against this receptor. Tirofiban, is among the list of that drugs which are formed against DC-61, to control bleeding. One of them is Tirofiban, which is drug used to prevent clotting ⁹.

To look into the effect of different drugs on different substances of body, drug designing and bioinformatics are used as primary tools. And different docking softwares are present for to probe the docking of drugs to molecule. In our study we used Patchdock and Firedock which are online docking softwares. Autodock vina was also used in our study. Ligand and receptor docking can be assessed through these docking softwares. Docking scores were used to look into the docking appropriateness ¹⁰⁻¹³.

Food and Drug Association (FDA) has insisted to look deeply into effects of PRT on different molecules ¹⁴. PEN-110 is mainly used in red cell bags, but it can affect the platelets in the blood after transfusion. Keeping in view the above mentioned observations, in the current study, we selected CD-61 as the target to find the docking of PEN-110. The aim of this study was to review the docking of PEN-110 at CD-61 which would give the idea about the effect of PEN-110 on function of platelets.

METHODOLOGY

The observational study was carried out in-silico at Medical College, Taif University, Taif, Saudi Arabia from March 1st, 2016 till September 30th, 2016. This was computational study for
TABLE - I: 2vdm HYDROGEN BONDS, HYDROGEN BONDS LENGTH AND HYDROPHOBIC BONDS

<table>
<thead>
<tr>
<th>Hydrogen Bonds</th>
<th>Bonds Length Å (Oxygen/Nitrogen)</th>
<th>Hydrophobic bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>2vdm Tirofiban with CD-61 bonds</td>
<td>Ser 225(A), 2.98</td>
<td>Asp 159(A), Phe 160(A), Tyr 189(A), Leu 192(A), Asp 224(A), Phe 231(A)</td>
</tr>
<tr>
<td>Ser 121(B), 2.77</td>
<td>Asp 216(B), Arg 217(B), Ala 218(B), Glu 220(B)</td>
<td></td>
</tr>
<tr>
<td>Tyr 122(B), 3.14</td>
<td>Ser 123(B), 2.86/2.70</td>
<td></td>
</tr>
<tr>
<td>Arg 214(B), 3.08</td>
<td>Asn 215(B), 2.76/2.55</td>
<td></td>
</tr>
</tbody>
</table>

It was found that best pose of CD-61 and Tirofiban docking through Patchdock didn't show any amino acids with which hydrogen bonds were formed, matching to the active side amino acids. Although Phe 160(A), Arg 214 (B), Arg 216(B) and Ala 218(B) were found to be same as active site as far as hydrophobic bonds are concerned. Hydrophobic results with Phe 160(A), Arg 214 (B) and Ala 218(B) were also found to be matching with active sites amino acids in Firedock results of best pose of CD-61 and Tirofiban. These details are given in Table - II. One out of top 20 Firedock docking results of PEN-110 to CD-61, were found to be overlapping with 2vdm tirofiban active site. So they were selected as best pose and named as best pose 1 for Firedock. No Patchdock docking result showed docking at active site. Arg 214(B) formed hydrogen bond with tirofiban in both Patchdock docking of Best pose 1 and 2. 57% of amino acids which formed Hydrophobic bonds with Tirofiban were same as active site amino acids in best pose 1 of Patchdock results. While in Best pose 2 of Patchdock results, 62.5% of amino acids were same as of active site.

One best pose in Firedock docking results of PEN-110 to CD-61, showed 1 hydrogen bond. But it was not found matching with the active sites amino acids. Although Hydrophobic bonds amino acids showed 33.3% homology with active site's amino acids.
Docking Scores of Patchdock docking of CD-61 with Tirofiban is given in Table III. No docking result of Patchdock showed attachment to active site.

Docking Scores of Firedock docking of CD-61 with Tirofiban and PEN-110 is given in Table IV. Docking scores were markedly greater for docking results of CD-61 and Tirofiban than for docking results of CD-61 and PEN-110. In contrast, Attractive van der wal forces, repulsive van der wal forces and ACE score were lesser for docking results of CD-61 and Tirofiban than for docking results of CD-61 and PEN-110.

Autodock vina docking scores and percentage difference with control are mentioned in Table V. Percentage docking difference from control was calculated. Then, Firedock, Patchdock and Autodock vina docking percentages were added and average was found, as shown in Table VI.

### TABLE - II: HYDROGEN BONDS, HYDROGEN BONDS LENGTH AND HYDROPHOBIC BONDS OF DOCKING RESULTS

<table>
<thead>
<tr>
<th>Hydrogen Bonds</th>
<th>%age similarity to 2VDM</th>
<th>Bonds Length Å (Oxygen/Nitrogen)</th>
<th>Difference from 2VDM</th>
<th>Hydrophobic bonds</th>
<th>%age similarity to 2VDM (A and B Chain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patchdock CD-61 with Tirofiban Best pose (A Chain)</td>
<td>0 %</td>
<td>0</td>
<td>0</td>
<td>Phe 160(A) Tyr 190(A) Phe 231(A) Asp 232(A) Trp 262(A)</td>
<td>40%</td>
</tr>
<tr>
<td>Patchdock CD-61 with Tirofiban (B Chain)</td>
<td>Tyr 166(B)</td>
<td>0 %</td>
<td>2.78</td>
<td>0</td>
<td>Tyr 122(B) Ser 123(B) Arg 214 (B) Arg 216(B) Ala 218(B)</td>
</tr>
<tr>
<td>Firedock Docking of CD-61 with Tirofiban Best pose (A Chain)</td>
<td>Tyr 166(B)</td>
<td>0 %</td>
<td>2.37</td>
<td>0</td>
<td>Arg 214(B) Ala 218(B)</td>
</tr>
<tr>
<td>Firedock Docking of CD-61 with Tirofiban (B Chain)</td>
<td>ASP 159(A)</td>
<td>0 %</td>
<td>2.87</td>
<td>0</td>
<td>Asn 158(A) Phe 160(A) Ser 161(A) Trp 162(A) Asp 224(A) Ser 225(A) Ser 226(A)</td>
</tr>
</tbody>
</table>

### TABLE - III: PATCHDOCK DOCKING SCORES

<table>
<thead>
<tr>
<th>Solution No.</th>
<th>PEN</th>
<th>SCORE</th>
<th>Area</th>
<th>ACE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patchdock CD-61 with Tirofiban Best pose</td>
<td>14.0</td>
<td>-2.2</td>
<td>5390.0</td>
<td>617.8</td>
</tr>
</tbody>
</table>

### TABLE - IV: FIREDOCK DOCKING SCORES

<table>
<thead>
<tr>
<th>Soln no.</th>
<th>Ranking</th>
<th>Global Energy</th>
<th>Attractive Vander wal forces</th>
<th>Repulsive Vanderwal forces</th>
<th>ACE</th>
<th>Inside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firedock CD-61 with Tirofiban Best pose</td>
<td>8.0</td>
<td>11.0</td>
<td>-5.99</td>
<td>-5.64</td>
<td>0.97</td>
<td>-2.67</td>
</tr>
<tr>
<td>Firedock Docking of CD-61 with PEN-110 Best pose</td>
<td>5.0</td>
<td>17.0</td>
<td>101.9</td>
<td>-23.38</td>
<td>192.12</td>
<td>-10.53</td>
</tr>
</tbody>
</table>

### TABLE - V: AUTODOCK DOCKING SCORES OF PATHOGEN REDUCING COMPOUNDS WITH CD-61:

<table>
<thead>
<tr>
<th>Autodock Scores</th>
<th>%Age Docking In Comparison To Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tirofiban</td>
<td>-7.8</td>
</tr>
<tr>
<td>PEN-110</td>
<td>-6.1</td>
</tr>
</tbody>
</table>
TABLE VI: AVERAGE PERCENTAGE DOCKING

<table>
<thead>
<tr>
<th></th>
<th>Patchdock docking</th>
<th>Firedock docking</th>
<th>Autodock vina docking</th>
<th>Average Percentage docking</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEN-110</td>
<td>0</td>
<td>-17</td>
<td>78</td>
<td>20.33</td>
</tr>
</tbody>
</table>

DISCUSSION

Safety in patient treated with blood products treated with Pathogen reduction compounds including PEN-110 is real concern in transfusion medicine. To find out the effect of PEN-110 on CD-61, in this study, Tirofiban and PEN-110 were docked to CD-61 through Patchdock and Firedock online docking softwares. 2vdm (CD-61 and Tirofiban complex) was used as control for comparison of docking results. Details of amino acids with which Hydrogen bond are formed, Hydrogen bonds length and amino acids with which Hydrophobic bonds are formed, of 2vdm, is given in Table I. These amino acids were considered as active site amino acids. The Hydrogen bonds and Hydrogen bond length and Hydrophobic bonds of docking results were then compared to 2vdm.

Best parameter to determine docking is the binding energy. In this study, PEN-110 showed binding to active site of CD-61 but with lesser binding force than binding of Tirofiban with CD-61. Docking score in Patchdock docking of CD-61 and Tirofiban best pose is 5690. While there was no docking observed for PEN-110 with CD-61. In Firedock docking, Global energy is significantly lower (P<0.005) in PEN-110 docking with CD-61 than with Tirofiban docking with CD-61. Attractive van der waal forces and ACE score was higher for docking of CD-61 to PEN-110 than CD-61 with Tirofiban. While repulsive van der waal forces are higher in PEN-110 docking with CD-61. These findings are recommending poor docking in case of PEN-110 with CD-61. Hydrogen bonds are quite important in determining the docking of drug to receptor. In this study we considered Hydrogen bonds of template structure 2vdm as a standard and then compared the Hydrogen bonds of docking results best poses with them. In the Patchdock and Firedock docking of CD-61 with Tirofiban, Tyr 166(B) showed hydrogen bond, but it was not an amino acid of active side. In the Patchdock docking of PEN-110 with CD-61, no binding at active site was observed. In Firedock docking of PEN-110 with CD-61, best pose had 1 Hydrogen bonds, but not with active side amino acid.

Hydrophobic bonds have importance in docking. It is stated that increase in hydrophobic bond in the ligand and active site interface helps to maximize effect of ligand. Similarly increase in hydrophobic bonds enhances the side effects of drug. In our study, Asp 159(A), Phe 160(A), Tyr 189(A), Leu 192(A), Asp 224(A), Phe 231(A),Arg 216(B), Asp 217(B), Ala 218(B), Glu 220(B) were found to be involved in Hydrophobic bonds in 2vdm. So, these were amino acids of active site which were involved in hydrophobic bonds. In Patchdock docking of CD-61 with Tirofiban 40% of amino acids which formed Hydrophobic bonds were identical to amino acids which formed hydrophobic bonds in 2vdm. In Firedock docking of CD-61 with Tirofiban, 50% of amino acids were found same as of 2vdm hydrophobic bond’s amino acids. In Firedock docking of PEN-110 with CD-61, 33.3% of amino acids showed similarity to amino acids of 2vdm which formed hydrophobic bonds.

Autodock vina docking showed relatively better docking score in comparison to Firedock and Patchdock docking. But it was still 78% in comparison to docking scores of control. We also did a comparison between the docking done by Patchdock and Firedock. The docking results of both softwares were quite similar. This comparison was only possible with docking of CD-61 and Tirofiban, as there was no docking seen in patchdock for CD-61 and PEN-110. Same amino acid Tyr 166(B) was docked in both Patchdock and Firedock docking of CD-61 and Tirofiban. Similarly, 40% and 50% of amino acids were found identical to active site in Patchdock and Firedock respectively.

In Patchdock, Firedock and Autodock vina dockings, docking results of CD-61 and Tirofiban docking showed more docking energy than docking of PEN-110 with CD-61. Only major difference among docking results of Patchdock and Firdock is that ACE (Atomic Contact Energy) score of Patchdock docking are lesser for PEN-110 with CD-61 than CD-61 and Tirofiban, in concordance with docking score. Overall average of docking percentage difference from control is 20%, which shows the weak docking of PEN-110 with CD-61.

CONCLUSION

PEN-110 binds to the active site of CD-61 with weaker binding force. We can assume that PEN-110 does not effect this important receptor of platelet which is needed for proper function of platelets.

DISCLAIMER: None.

CONFLICT OF INTEREST: None.

SOURCE OF FUNDING: None.

REFERENCES

6. Schlenke P. Pathogen Inactivation Technologies for Cellular
16. PyMOL 0.99rc6 [Internet]. Malaga, Spain: DeLano Scientific LLC; 2006 [cited date: June 15th 2016]. Website: [http://pymol.en.uptodown.com/]
18. PDBSum [Internet]. UK:  EMBL-EBI; 1992 [cited date: June 20th 2016]. Website: [http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html]
19. Protein Data Bank in Europe. UK:  EMBL-EBI; 1992 [cited date: June 20th 2016]. Website: [www.ebi.ac.uk./pdbe-site/pdbemotif]