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IN-SILICO ANALYSIS;

ANALYSIS OF S-303 BINDING TO CD-61 OF PLATELETS

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ABSTRACT… Introduction: Different pathogen reducing technologies are being implemented which includes S-303. CD-61 is important receptor for clotting. Pathogen reducing agents are being studied extensively to probe its effects. **Objective:** We conducted this study to review the docking of S-303 at CD-61, to look into the effect of S-303 on function of platelets. **Study Design:** This was an observational study. **Setting:** In-silico study. **Period:** March 2015 to August 2015. **Method:** The study was carried out in-silico. PDB (Protein data bank) code of Tirofiban bound to CD-61 was 2vdm. CD-61 was docked with Tirofiban using online docking tools i.e. Patchdock and Firedock. Then, S-303 and CD-61 were also docked. Best docking poses to active sites of 2vdm were found. Interactions of ligands and CD-61 were obtained. Then comparison of Hydrogen Bonds, Hydrogen Bond Lengths, Hydrophobic bonds of 2vdm molecule and best poses of docking results were done. Patchdock and Firdock results of best poses were also analyzed using SPSS-16. **Results:** The Hydrogen bonds and Hydrogen bond length and hydrophobic bonds of docking results were compared to 2vdm. 2 best poses were obtained for docking of tirofiban to CD-61. No docking to active site was observed in Patchdock and firedock for S-303to CD-61. **Conclusion:** S-303 did not bind to the active site of CD-61. We can assume that S-303 does not affect this important receptor of platelet which is needed for proper function of platelets.

Key words: S-303, CD-61, Platelets.

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STATEMENT OF NOVELTY

Pathogen reducing agents are being studied extensively to probe its effects. We conducted this study to review the docking of S-303 at CD-61, to look into the effect of S-303 on function of platelets. There is no published study on this aspect of relationship between S-303 and platelets.

INTRODUCTION

Blood Transfusion is salient feature of the patient care. One of the biggest aims to be achieved is to make the transfusion safe. HIV, Hepatitis B and Hepatitis C have been prioritized always regarding blood transfusion. This was the reason that in last ten years, viral screening and nucleic acid testing have been considered the top most tools to reduce the chances of transmission of virus.[1] In spite of all these efforts infections like viruses, protozoan's, helminthes, prions and bacteria have not been controlled as far as transfusion is concerned.[2] Platelet bags are always been considered notorious due to growth of bacteria. Storage of platelet bags at room temperature and the biological condition of bag, are the key reasons for growth of bacteria in platelet bags.[3] Rate of platelet bag contamination which has been calculated is 1/2,000-3,000 units.[4] It has also been observed that ratio of severe sepsis to infected transfusion is 1/6.[4]

Pathogen reducing technology (PRT) has been implemented since last few years, to control growth of bacteria in blood transfusion bags. Among them the dyes which have been studied are riboflavin, the essential vitamin B2, and the phenothiazine derivative methylene blue, psoralens, such as S-59 (amotosalen-HCl) and S-303 or the ethylene imine PEN-110.[5,6]

S-303, one of the PRT, is an acridine nitrogen mustard alkylating agent. Studies have mentioned that S-303 leads to inactivation of pathogens with preservation of RBC properties. But antibody formation is reported against RBC. It is suggested in many studies to test this S-303 in whole blood components. Many studies recommend to probe different aspects for S-303.[7]

CD (Cluster of differentiation)-61 is one of the main antigens on platelets, which helps in clotting of blood. It does so by binding to fibrinogen. Due to this reason, several antagonists have been developed against CD-61 to control the clotting of blood. One of them is Tirofiban, which is drug used to prevent clotting.[8]

Drug designing and bioinformatics are used primarily as a tool to probe the effects of drugs on different molecules of body. There are several docking software's available for this purpose. Patchdock and Firedock are also one of the online docking software's, which were used in our study. These docking software's help to dock the ligand and molecule and give several docking possibilities. They calculate docking scores which aid in deciding the appropriate docking of the ligand and molecule.[9,10]

Keeping in view the above mentioned observations, in the current study, we selected CD-61 as the target to find the docking of S-303. The aim of this study was to check the in-silico docking of S-303 to CD-61, to find whether S-303 can affect the CD-61which can lead to effects on platelet functions or not, because S-303 has been tried in whole blood component. FDA has also in enforced to find details of different aspect of S-303.[11]

MATERIAL AND METHODS

The study was carried out in-silico. Literature was searched for antagonists of CD-61(Glycoprotein IIIa/GPIIIa) on Human platelets. It was found that Tirofiban was antagonist to CD-61.PDB (Protein data bank) code of, CD-61 bound with tirofiban, was found through PDBsum, which was 2vdm. PDB structure of 2vdm was downloaded through website http://www.rcsb.org/pdb/exploredo?structureId=2vdm.Ligplot of interactions of CD-61 and Tirofiban (2vdm) was downloaded from PDBsum. Tirofiban and other ligands attached to CD-61 were removed by using Pymol software. Also Tirofiban molecule was removed from 2vdm structure and saved as PDB format. Then CD-61 (from which ligands were removed) was docked with Tirofiban (which was removed from 2vdm) using Patchdock and Firedock. Patchdock and Firedock are both online docking tools. PDB files of CD-61 and Tirofiban were deposited online and docking results were received through email.

S-303 (Pubchem CID:493570) SDF structure was downloaded through website http://pubchem.ncbi.nlm.nih.gov/. SDF file was converted to PDB file using Pymol software. Pymol is software for the analysis of interactions between the ligand and protein complex, (http://pymol.en.uptodown.com/). S-303 and CD-61 were docked by using Patchdock and Firedock.

20 best solutions given by Patchdock and Firedock docking results were assessed through Pymol. 2vdm was used as control and docking of Tirofiban and S-303 to CD-61were overlapped with Tirofiban in 2vdm, to find the binding of ligands (Tirofiban and S-303) to active sites. The best poses (which were best in binding grossly to active sites in pymol) were selected. PDB file of these best poses were submitted to http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html and www.ebi.ac.uk./pdbe-site/pdbemotif/. Ligplot of interactions of ligands and CD-61 were obtained. Then comparison of Hydrogen Bonds, Hydrogen Bond Lengths, Hydrophobic bonds of 2vdm molecule and best poses of docking results were done. Patchdock and Firdock results of best poses were also analyzed using SPSS-16.

RESULTS

Docking of best pose (ligands binding to active sites) of Tirofiban and S-303to CD-61 were done through Patchdock and Firedock online docking softwares. The Hydrogen bonds and Hydrogen bond length and hydrophobic bonds of docking

results were then compared to 2vdm (CD-61 bound with Tirofiban). Details of amino acids with which Hydrogen bonds 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.

It was found that best pose of CD-61and 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 bonds 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-61and Tirofiban.

None out of top 20 Firedock docking results of S-303 to CD-61, were found to be overlapping with 2vdm tirofiban active site. Similarly, no Patchdock docking result showed docking at active site.

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 is given in Table IV.

DISCUSSION

Safety in patient treated with blood products treated with Pathogen reduction compounds including S-303 is real concern in transfusion medicine.

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

Table-I. 2vdm hydrogen bonds, hydrogen bonds length and hydrophobic bonds

	Hydrogen Bonds	%age similarity to 2VDM	Bonds Length Å (Oxygen/Nitrogen)	Difference from 2VDM	Hydrophobic bonds	%age similarity to 2VDM (A and B Chain)
Patchdock CD-61 with Tirofiban Best pose (A Chain)		0 %	0	0	Phe 160(A) Tyr 190(A) Phe 231(A) Asp 232(A) Trp 262(A)	40%
Patchdock CD-61 with Tirofiban (B Chain)	Tyr 166(B)	0 %	2.78	0	Tyr 122(B) Ser 123(B) Arg 214 (B) Arg 216(B) Ala 218(B)	
Firedock Docking of CD-61 with Tirofiban Best pose (A Chain)					Phe 160(A) Tyr 190(A) Pro 228(A) Phe 231(A)	50%
Firedock Docking of CD-61 with Tirofiban (B Chain)	Tyr 166(B)	0	2.37	0	Arg 214(B) Ala 218(B)	

Table-II. Hydrogen bonds, hydrogen bonds length and hydrphobic bonds of docking results

	Solution No.	PEN	SCORE	Area	ACE
Patchdock CD-61 with Tirofiban Best pose	14.0	-2.2	5390.0	617.8	-152.88

Table-III. Patchdock docking scores

	Soln no.	Ranking	Global Energy	Attractive Vander wal forces	Repulsive Vanderwal forces	ACE	Inside
Firedock CD-61 with Tirofiban Best pose	8.0	11.0	-5.99	-5.64	0.97	-2.67	8.51

Table-IV. Firedock docking scores

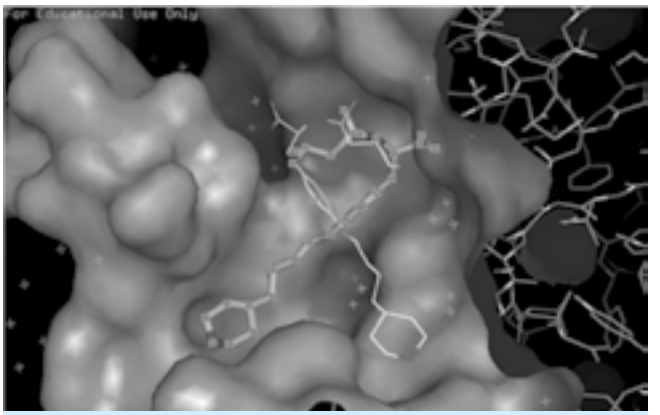

Figure-1. Best pose of patchdock docking of CD-61 and tirofiban
LIGHT GREY=TIROFIBAN OF 2VDM
WHITE=DOCKED TIROFIBAN

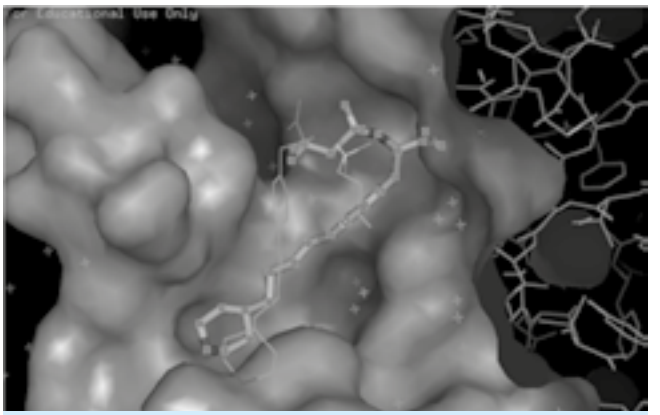

Figure-2. Best pose of patchdock docking of CD-61 and tirofiban
LIGHT GREY=TIROFIBAN OF 2VDM
LIGHT GREY=DOCKED TIROFIBAN

To find out the effect of S-303on CD-61, in this study, Tirofiban and S-303 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

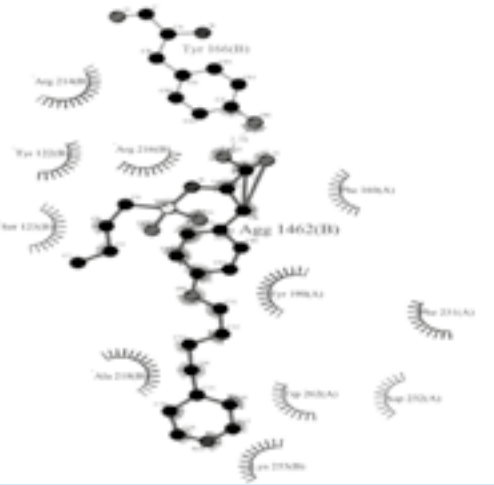

Figure-3: Ligplot of best pose of patchdock Docking of CD-61 with tirofiban

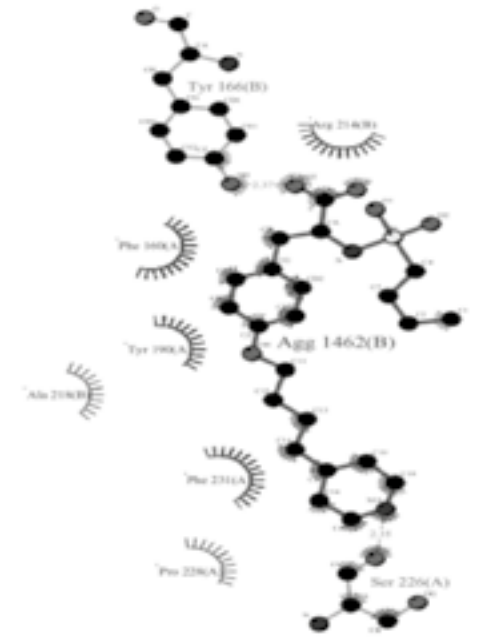

Figure-4. Ligplot of best pose of firedock docking of CD-61 with tirofiban

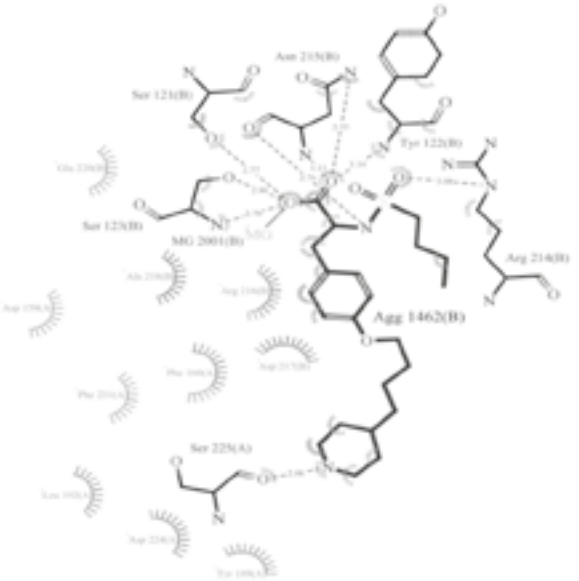

Figure-5. Ligplot of 2vdm cd-61 with tirofiban

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.[9,10,12] Docking score in Patchdock docking of CD-61 and Tirofiban best pose is 5690. While there was no docking observed for S-303 with CD-61.In Firedock docking, Global energy is -5.99 for Tirofiban and CD-61 best pose. But, no docking to the active site was observed for CD-61 and S-303 docking. These findings are recommending lack of docking in case of S-303 with CD-61.

Hydrogen bonds are quite important in determining the docking of drug to receptor.[13] 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 and firedock docking of S-303 with CD-61, no binding at active site was observed.

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.[13] 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 and Patchdock docking of S-303with CD-61, no docking was observed to active site of CD-61.

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 S-303. 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.

CONCLUSION

S-303 did not bind to the active site of CD-61. We can assume that S-303 does not affect this important receptor of platelet which is needed for proper function of platelets, although it needs further studies for confirmation of this finding.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Hammad Tufail Ch.	Designing of the work, acquisition, analysis interpretation of data and drafting the work.	
2	Dr. Shahida Hasnain	Designing, analyzing, revising it critically for important intellectual content and proof reading the study.