DEVELOPMENT OF COMPETITIVE POTENT DRUG TARGET TO FACTOR VIII IN HAEMOPHILIA

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ABSTRACT
Hemophilia A is a hereditary bleeding disorder caused by a lack of blood clotting factor VIII. It is caused by hundreds of different mutations and manifests itself in clinical conditions of varying severity [8]. This study is an attempt to design such a drug which controls the blood clotting during injury and to maintain the biological function of coagulation factor VIII. By using several tools, a drug library is created and they are tested for their ADMET and TOPKAT properties. The qualifying drug is selected for docking with receptor molecule 2R7E. It gives good binding site towards the active site of the receptor which is used to stop bleeding when a blood vessel is broken. This study may pave a new way for better treatment for hemophilia.

Key words: Pharmacophore, docking, minimization, coagulation factor, Lipinski rule.

INTRODUCTION
Haemophilia is a group of hereditary genetic disorders that impair the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken. Persons with haemophilia may bleed for a longer time than others after an injury or accident [11]. They also may bleed internally, especially in the joints (knees, ankles, and elbows). Babies born with haemophilia are missing or have a low level of a protein needed for normal blood clotting or blood coagulation [2]. The protein is called coagulation Factor XIII [3]. The major types of this condition are hemophilia A (also known as classic hemophilia) they are caused by mutations in different genes. These genes are located on the X chromosome.

Coagulation factor VIII
Recommended name for this protein is Coagulation factor VIII and Alternative name Antihemophilic factor, Procoagulant component. (www.uniprot.org)
This protein circulates in the bloodstream in an inactive form, bound to another molecule called von Willebrand factor, until an injury that damages blood vessels occurs. In response to injury, coagulation factor VIII is activated and separates from von Willebrand factor. The active protein interacts with another coagulation factor called factor IX. This interaction sets off a chain of additional chemical reactions that form a blood clot. Coagulation factor VIII is made chiefly by cells in the liver.

F8 Gene in genomic location: bands according to Ensembl, locations according to Geneloc

But the Mutations in the F8 gene lead to the production of an abnormal version of coagulation factor VIII or reduce the amount of this protein[10]. The altered or missing protein cannot participate effectively in the blood clotting process. As a result, blood clots cannot form properly in response to injury. These problems with blood clotting lead to excessive bleeding that can be difficult to control. Some mutations, such as the large inversion described above, almost completely eliminate the activity of coagulation factor VIII and result in severe hemophilia. Other mutations reduce but do not eliminate the protein's activity, resulting in mild or moderate hemophilia. The length of protein sequence is 2351 amino acid. The main function of this protein is to attach carbohydrate-binding domain to the cell surface, present in eukaryotes and assumed to have horizontally transferred to eubacterial genomes

Metabolic pathway
This gene encodes coagulation factor VIII, which participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca+2 and phospholipids, converts factor X to the activated form Xa. This gene produces two alternatively spliced transcripts. Transcript variant 1 encodes a large glycoprotein, isoform a, which circulates in plasma and associates with von Willebrand factor in a noncovalent complex. This protein undergoes multiple cleavage events. Transcript variant 2 encodes a putative small protein, isoform b, which consists primarily of the phospholipids binding domain of factor VIIIc. This binding domain is essential for coagulant activity. Defects in this gene results in hemophilia, a common recessive X-linked coagulation disorder.(www.genome.jp/kegg/pathway.)

MATERIALS AND METHODS

Bioinformatics represents a new, growing area of science that uses computational approaches to answer biological questions. The potential of such an approach is beginning to change the fundamental way in which basic science is done, helping to more efficiently guide experimental design in the laboratory. With the explosion of sequence and structural information available to researchers, the field of bioinformatics is playing an increasingly large role in the study of fundamental biomedical problems.

Databases used:-
- PDB(www.pdb.org)
- Gene Cards (www.genecards.org)
- Pubchem(www.ncbi.nlm.nih.gov/pccompound)
- Drug bank (www.drugbank.ca)
- UNI-PROT(www.uniprot.org)

Actual method:-
The coagulation factor VIII which is the target protein of length 2351 amino acid was retrieved from NCBI database and it was validated from gene card. The sequence analysis of target protein was done with 10 model organism by using BLAST. The structure analyses of target protein was studied by using cph model3.0 Server, to retrieved the receptor molecule Then validation of target protein sequence was done by using various
confirmatory tools like ProDom, Pfam, CATH, Prosite, SOSUI etc. Receptor was minimized by using Accelyrs Discovery Studio 2.5. Active site of receptor is identified by using online tools like PDB SUM, Q SITE finder. Six ligands were designed with help of CHEMSKETCH TOOL and drug library was created. All the ligand compounds were passed through ADMET & TOPKAT filter in Accelyrs Discovery Studio2.5. Non toxic and non carcinogenic ligand compounds are selected for docking with receptor molecule by using Accelyrs Discovery Studio 2.5 and HEX6.3.

RESULT AND DISCUSSION
The structure analysis of target protein was studied by using CPH model 3.0Server. The protein databank identification number (PDB id) 2R7E was selected, having least E value i.e.0.0 and high score value i.e. is 1449 to retrieved the receptor molecule from PDB (www.pdb.org)

CPH MODEL 3.0Server
Searching for template ...
Round 0. Hits better than threshold: 0.000010:
entry: 2R7E chain: A score: 1449 E: 0.0
entry: 2R7E chain: B score: 1370 E: 0.0
entry: 3CDZ chain: B score: 1136 E: 0.0
entry: 3CDZ chain: A score: 1293 E: 0.0
entry: 1SDD chain: B score: 520 E: 1e-146
entry: 275W chain: A score: 425 E: 1e-118
entry: 1KCE chain: A score: 422 E: 1e-117
entry: 1D7P chain: M score: 334 E: 9e-91
entry: 3HNB chain: M score: 330 E: 1e-89

Receptor Minimization
Receptor is prepared for minimization by removing water molecules, hetro atom and minimized by applying force field CHARMM in Accelyrs Discovery Studio 2.5. Active site of receptor is identified by using online tools like PDB SUM, Q SITE finder. Thus minimization was obtained with constant energy -91165.8864 kcal/mol at conjugant gradient 1800 in fig:2

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Table.1 drug library with there molecular properties

ADMET & TOPKAT
All the ligand compounds were passed through the ADMET/TOPKAT filters to discard the toxic and carcinogenic compounds in Accelyrs Discovery Studio2.5 ADMET have many different
parameters which give us information about the toxicity and TOPKAT gives us the information about the carcinogenicity of compounds. Non toxic and non carcinogenic ligand compounds L.4 and L.5 were further proceed for docking.

<table>
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<tr>
<th>Ligand</th>
<th>Admet_sol</th>
<th>Admet_olv</th>
<th>Admet_lip</th>
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Table.2 shows the ADMET analysis

Docking in Accelyrs Discovery Studio 2.5
Accelyrs Discovery Studio 2.5 is used for predicting the receptor-ligand interactions. The process of docking involves a conformational search for a compound which complements a target binding site, with the aim of identifying the best matching binding pose. Here, the ligand compound L.5 gives best 10 binding pose with least Cdocker energy i.e. **-112.46** fig.5

Docking in HEX 6.3
Hex docking is done to performed pharmacophore and to calculate the E-mini and E-max value. E-mini = -157.46, E-max = -131.34.

Table.3 docking result.
Ligandscout

Ligandscout is used to study the pharmacophore of protein-ligand complex. In the result red color shows ionic properties, and binding sites which are attached to arginine and histidine.

CONCLUSION

The receptor-ligand interactions play a significant role in molecular docking and drug designing. The receptor 2R7E interacts with the ligand compound \((2S)-2'-\text{amino}-5-(\text{aminomethyl})-4'-\text{hydroxy}-1,1'\text{-bi(cyclohexyl)}-2-\text{carboxylic acid}\) effectively with the best minimum energy -112.46., which may be more potent then the approved drug.

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