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Research Article

SCREENING OF COMPETITIVE INHIBITOR OF HEPARAN SULFATE IN JAPANESE ENCEPHALITIS

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ABSTRACT

Objective: Japanese encephalitis virus (JEV) causes central nervous system inflammatory disease Japanese encephalitis (JE), which is mainly caused in children below 15 years of age. On an estimate, there are around 3 billion people at the risk and the disease is continuously spreading globally. The JEV belongs to *Flavivirdiae* family and has RNA genome. JEV envelope protein domain III (D-III) binds to the Heparan sulfate present on the cell surface and initiates the infection which causes the disease in children.

Methods: The drug discovery and development process has become more quantitative and much more computational in recent years. In this study, comparative molecular docking studies of 200 zinc database compounds and Heparan sulfate were done with D-III of JEV using Autodock 4.2 and the results were analyzed on the basis of binding energy, inhibition constant, and number of hydrogen bonds. The results were also analyzed by studying the absorption, distribution, metabolism, and excretion (ADME-T) properties of the compounds using admetSAR server.

Results: Best three lead molecules zinc_8964844 zinc_8964845, zinc_12660861 were chosen based on the binding energy, inhibition constant and ADME properties among a set of 200 ligands that can act as the competitive inhibitor of the Heparan sulfate, which presents on the surface of the host cell and mediates the attachment and binding of the virus to the host cell.

Conclusion: These compounds can act as the competitive inhibitor of the Heparan sulfate and they can be validated further as a drug for the treatment of JE.

Keywords: Japanese encephalitis, Domain-III, Heparan sulfate, Autodock, Autodock, Absorption, Distribution, Metabolism, Excretion.

INTRODUCTION

Japanese encephalitis virus (JEV) causes JE, which is a leading form of viral encephalitis in Asia, with around 50,000 cases and 10,000 deaths per year in children below 15 years of age [1]. It is transmitted by the *Culex* mosquitoes between wild and domestic bird and pigs [2] and is the main cause of encephalitis in eastern and southern Asia [3]. The cycle involves water bird as carrier, pigs as the major reservoir/amplifying host, mosquitoes as vectors and human as dead end host because of low viremia levels [4]. JEV belongs to the family *Flaviviridae* and genus *Flavivirus* [5]. It is a single stranded, positive-sense polarity RNA genome of approximately 11 kb in length. The virion of JEV contains three structural proteins – nucleocapsid or core protein (C), non-glycosylated membrane protein (M), and glycosylated envelope protein (E), as well as seven non-structural (NS) proteins – NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS [6].

JEV E-protein has three domain organization and are connected by a disulfide bond. The central domain I (D-I) have nine-stranded β-barrel situated between the extended domain III (D-II) and globular domain III (D-III). D-II is formed of two extended loops and protrude out from D-I, the larger the loop stabilized by three disulfide bonds and have conserved fusion peptide loop at the tip while DIII have an immunoglobulin like fold and is found at the C-terminus of the ectodomain connected by a short linker to D-I [7].

The D-III conformation, structure is maintained by 1 disulfide bond and it can fold independently as trypsin-resistant core protein. The lateral surface of DIII contains neutralizing epitopes. Mutations on DIII can lead to the escape from antibody neutralization. JEV DIII forms β-barrel like structure having six antiparallel β-strands similar to immunoglobulin constant domain. The top of β-barrel is closed by N-terminal residues and loops between the β2-β3, β3-β4 and β5-β6 strands. Among all the three structural domains, DIII is the chief antigenic domain of E-protein [8]. The DIII domain interacts between the primary and secondary cell surface receptors, including Heparan sulfate and ribosomal proteins, and virus [9].

There are many groups of vaccine like purified, formalin-inactivated mouse-brain derived, cell culture derived, inactivated and cell culture derived live attenuated, which are currently in use but there is no specific treatment against JEV strains. Studies identified various molecular targets for the *flavivirus* drug discovery: Envelope glycoprotein, NS3 protease, NS3 helicase, NS5 methyl transferase and NS5 RNA-dependent RNA polymerase [10]. In the present study, we have been trying to develop the small non-peptide molecule to inhibit the JEV attachment to the cell surface by searching for an inhibitor, which can bind to JEV D-III and prevent the binding of DIII to its natural

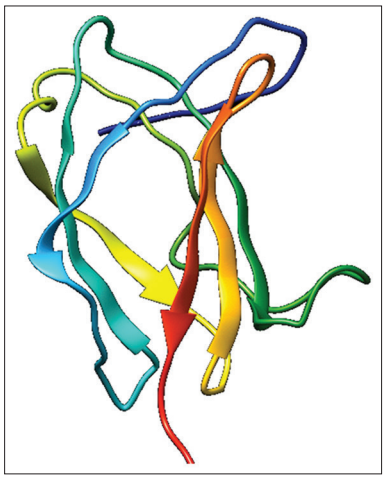

Fig. 1: Structure of domain-III of Japanese encephalitis virus (Protein Data Bank ID-1PJW)

effector molecule i.e., Heparan sulfate. An appropriate set of ligands was taken from ZINC database and the protein-ligand interaction was studied using Autodock 4.2 and the lead molecule was validated by absorption, distribution, metabolism, and excretion (ADME-T) studies.

COMPUTATIONAL METHODS

Retrieval of receptor

JEV envelope protein D-III structure was retrieved from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) server. The structure of JEV envelope protein D-III (PDB Id: 1PJW) was of 111 amino acid residues and had one chain without having any ligand as shown in Fig. 1. The structure was resolved using NMR and this structure was used for docking analysis.

Screening and preparation of ligands

The ligands were screened using RASPD [11] (http://www.scfbio-iitd.res.in/software/drugdesign/raspd2.jsp) server. Method B: Protein 3D Structure without ligand was used and the natural product database selected with three available methods, i.e., Million Molecule Database, Natural Product Database, and NCI Database keeping all the parameters default. Out of 427248 zinc compound given by the server, a set of 200 compounds was chosen. The compounds were downloaded from the zinc database [12] (http://zinc.docking.org/) in the *.sdf* format. PubChem (https://pubchem.ncbi.nlm.nih.gov/) Database was used to download the structure of Heparan sulfate (CID: 53477714) shown in Fig. 2. The downloaded ligands were converted into.*pdb* format using the Openbable v2.3.2 [13]. Ligands were prepared by neutralization of charged groups and adding of the hydrogen bonds. These prepared ligands were finally used for docking using autodock 4.2.1.

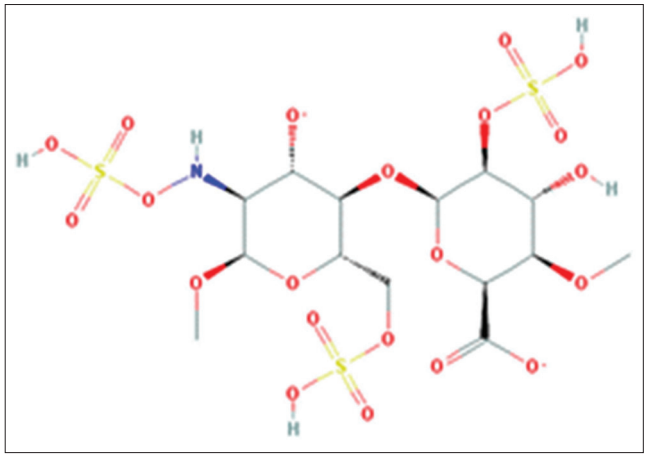

Fig. 2: Chemical structure of heparan sulfate

Binding site prediction

Active sites, binding sites, shape (alpha complex and triangulation), volume (solvent and molecular accessible surface), surface structural pockets (accessible), interior cavities (inaccessible) of every pocket and cavities were found using CASTp [14] server (http://sts.bioe.uic.edu/castp/calculation.php).

Molecular docking studies

The docking of the receptor protein (PDB id: 1PJW) with the suitable ligands and the Heparan sulfate was done using the autodock 4.2 [15]. Input files were prepared by adding polar hydrogen, Kolloman charges and setting up the grid map. Grid box parameters were set to 72Å × 90Å × 64Å with grid points separated by 0.375Å and center grid box offsets −1.194Å, −4.278Å, 1.611Å. Rigid docking was performed using a Lamarckian Genetic algorithm and the runs were increased to 100 to find out the most preferred orientation of the ligand to the receptor, having the lowest binding energy and finally two-dimensional image was generated using LigPlot [16].

ADME-T prediction

The ADME-T properties of the best ligands and Heparan sulfate were calculated using an admetSAR server [17] (http://lmmd.ecust.edu.cn:8000/) various properties of chemical compounds such as blood-brain barrier, human intestinal absorption, AMES toxicity, Carcinogenicity, and biodegradation were calculated using the server.

RESULTS AND DISCUSSION

Binding site analysis

It was necessary to identify the binding residues of D-III of JEV and therefore D-III constitutes LEU3, ALA4, LEU5, LYS6, GLY7, TYR10, MET12, PHE17, PHE19, LYS21, ASN22, PRO23, ALA24, ASP25, GLY29, VAL31, VAL32, ILE33, GLU34, LEU35, SER36, TYR37, SER38, GLY42, PRO43, LYS45, ILE46, ILE48, SER50, VAL51, ALA52, ASN55, ASP56, GLY61, ARG62, LEU63, VAL66, ASN67, PRO68, PHE69, VAL70, ALA71, THR72, ASN76, LEU80, VAL81, GLU82, MET83, PRO85, PRO86, PHE87, GLY88, ASP89, SER90, TYR91, ILE92, VAL93, VAL94, ILE101, ASN102, HIS103, TRP105, HIS106, LYS107 amino acids in the active site.

Virtual screening analysis

200 compounds were screened from Zinc database as process described earlier and selected compounds shown in Table 1 and best three compounds were selected which gives better binding energy and inhibition constant of that compared with Heparan sulfate shown in Table 2.

Table 1: List of 200 compounds docked with envelope protein domain III of JEV

8790643	8790676	8964810	8964840	8964867	11616528	11866944	12660876	11616365	12660914
8790644	8790677	8964811	8964842	8964868	11616531	11866945	12660878	11616366	12660915
8790645	8964782	8964812	8964843	8964869	11616537	11866946	12660881	11616367	12660916
8790646	8964783	8964818	8964844	8964870	11616539	11866947	12660886	11616368	12660917
8790647	8964784	8964819	8964845	8964871	11616540	11866948	12660887	11616398	12660953
8790649	8964785	8964820	8964846	8964876	11616541	11866949	12660889	11616399	12660954
8790653	8964786	8964821	8964847	8964877	11616542	11866950	12660893	11616400	12660879
8790654	8964787	8964822	8964848	8964878	11616543	11866951	12660894	11616401	11866940
8790656	8964788	8964823	8964849	8964879	11616545	11866952	12660896	11616402	11616550
8790659	8964789	8964824	8964851	8964880	11616555	11866953	12660897	11616403	8964806
8790660	8964794	8964825	8964853	8964881	11616556	11866954	12660898	11616404	12660862
8790662	8964795	8964826	8964854	8964882	11616557	12660858	12660899	11616405	12660956
8790663	8964796	8964827	8964855	8964883	11616558	12660859	12660902	11616465	12660958
8790664	8964797	8964829	8964856	8964884	11616559	12660860	12660907	11616470	12660961
8790667	8964798	8964834	8964857	8964885	11616560	12660861	12660908	11616472	3875150
8790669	8964799	8964835	8964858	8964886	11616561	12660863	12660909	11616507	5179475
8790671	8964704	8964836	8964862	8964887	11866939	12660864	12660910	11616508	5179479
8790673	8964705	8964837	8964863	8964888	11866941	12660865	12660911	11616517	8740013
8790674	8964707	8964838	8964864	8964889	11866942	12660866	12660912	11616526	8740509

JEV: Japanese Encephalitis Virus

ADME-T analysis

Selected compounds were then analysed for ADME-T properties using the admetSAR server and the result obtained are shown in Table 3.

The docking of D-III envelope protein of JEV was carried with the Heparan sulfate and zinc database compounds using the Autodock4.2 and the results were analysed on the basis of binding energy, inhibition constant and ADME properties. It can also be inferred from the study that some amino acid plays a major role in interaction between the ligands and the receptor which were asparagine 25, glycine 27, glycine 29 and lysine107. Out of set of 200 molecules docked, the best three ligands were selected on the basis of binding energy and inhibition constant and zinc_8964844 zinc_8964845, zinc_12660861 showed the better binding energy and the inhibition constant as that of compared to the D-III natural effector molecule i.e., Heparan sulfate shown in Figs. 3 and 4. These were non-carcinogenic and Non-AMES toxic as stated by the admetSAR server, as shown above in Table-4 and thus they can also inhibit the JEV infection during its attachment to the cell surface.

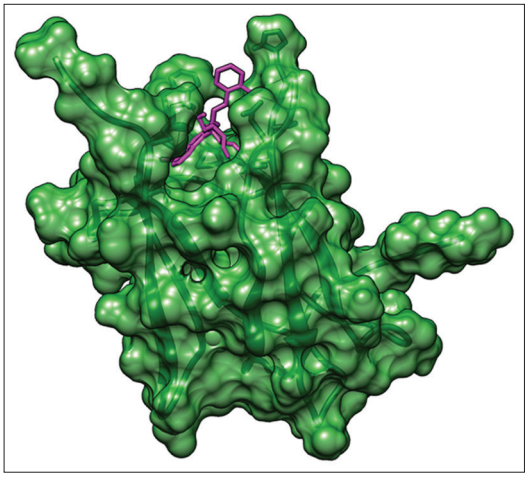

Fig. 3: Showing the ligand zinc_8964844 (magenta) in the cavity of the protein (green)

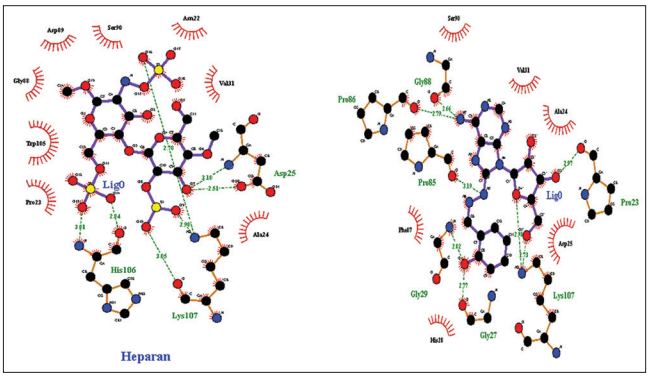

Fig. 4: Ligplot showing the interaction of heparan sulfate (left) and zinc_8694823 (right) with the protein

Table 2: Showing various parameter of docking of Heparan sulfate and selected compounds

Zinc ID	RMSD	Binding energy (kcal/mol)	No. of H-Bonds	Binding residues (single letter code)	Inhibition (µm)
Heparan sulfate	10.13	−5.12	7	N25, H106, K107	18.05
8964844	11	−7.69	8	P23, G25, P85, P86, G88, K107	2.3
8964845	11.65	−7.82	7	P23, N25.G27, G29, P86, P88, K107	1.84
12660861	10.13	−6.47	4	G27, H28, G29, K107	0.0305

RMSD: Root mean square deviations

Table 3: ADME properties of compound using the admetSAR server

Zinc Ids	BBB		HIA		$Caco_2$ Permeability		Renal organic cation transporter		CYP Inhibitory Promiscuity	
	Result	Probability	Result	Probability	Result	Probability	Result	Probability	Result	Probability
Heparan sulfate	BBB-	0.8851	HIA-	0.9868	$Caco_2$	0.6167	Non-Inhibitor	0.9369	Low CYP inhibitory Promiscuity	0.9165
8964844	BBB+	0.9134	HIA+	0.9805	$Caco_2$	0.7189	Non-Inhibitor	0.8626	Low CYP inhibitory Promiscuity	0.5632
8964845	BBB+	0.9134	HIA+	0.9805	$Caco_2$	0.7189	Non-Inhibitor	0.8626	Low CYP inhibitory Promiscuity	0.9451
12660861	BBB+	0.7351	HIA+	0.9297	$Caco_2$	0.7108	Non-Inhibitor	0.8134	Low CYP inhibitory Promiscuity	0.5632

HIA: Human intestinal absorption, BBB: Blood-brain barrier, CYP: Cytochrome P 450, ADME: Absorption, distribution, metabolism, and excretion

Table 4: Toxicity properties of compounds using admetSAR server

Zinc id	Human ether a-go-go-Related Gene Inhibition		AMES toxicity		Carcinogen		Biodegradation		Acute oral toxicity	
	Result	Probability	Result	Probability	Result	Probability	Result	Probability	Result	Probability
Heparan sulfate	Weakinhibitor	0.8687	Non AMES toxic	0.5725	Non-carcinogen	0.6354	Not readily biodegradable	0.7309	III	0.5612
8964844	Weak inhibitor	0.9431	Non AMES toxic	0.6722	Non-carcinogen	0.8375	Not readily biodegradable	1	III	0.6040
8964845	Weak inhibitor	0.9431	Non AMES toxic	0.6722	Non-carcinogen	0.8375	Not readily biodegradable	1	III	0.6040
12660861	Weak inhibitor	0.9803	Non AMES toxic	0.7844	Non-carcinogen	0.9584	Not readily biodegradable	0.9842	III	0.6095

CONCLUSION

Heparan sulfateplays a major role in attachment of JEV envelope protein to cell surface. In the present study it was revealed that zinc_8964844 zinc_8964845, zinc_12660861 compounds can act as the competitive inhibitor of the Heparan sulfate and can prevent binding of Heparan sulfate with the D-III and thus stop the infection of JEV as shown in Figs. 3 and 4, still a detailed study of the above compounds is needed by *in-vitro* and *in-vivo* methods of the compounds to prove the above fact.

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