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In Silico Analysis of Lutein and Rosmarinic Acid against Envelope Protein Domain III of Japanese Encephalitis Virus

Sahil Kulkarni[1], Lalit R. Samant[2*], Sneha Verma[3] and Abhay Chowdhary[1, 2,4]
[1] Department of Zoonosis, Haffkine Institute
[2] Systems Biomedicine Division, Haffkine Institute
[3]Departement of Biotechnology and Bioinformatics,
Dr. D.Y. Patil University, Plot no.-50, Sector-15, CBD Belapur, Navi Mumbai-400614
[4]Department of Virology and Immunology, Haffkine Institute
Address of correspondence -: Email ID: samantlalit@gmail.com -Telephone: +91 22 2416 0947 Fax: +91 22 2416 1787

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ABSTRACT

Japanese Encephalitis (JE) is a serious vector borne viral disease especially in South East Asia. Although preventable vaccines are available there is no known chemotherapeutic molecule available. The Japanese Encephalitis Virus (JEV) envelope domain III protein has been used in the past as experimental targets for developing chemotherapeutic molecules. The present study was aimed at *in silico* evaluation of known antivirals such as Lutein and Rosmarinic acid against JEV. The JEV envelope domain III protein structure retrieved from PDB was used as receptor. Six ligands selected from the ZINC Database were docked with the target protein using iGEMDOCK$^{V.2.10}$ docking tool. The analyzed results suggest that Rosamarinic acid ligands were found to be energetically more favorable making it more suitable for further in *in-vitro* analysis of anti JEV activity

Key words: Japanese Encephalitis Virus, Domain III, Docking, iGEMDOCK

INTRODUCTION

Encephalitis means an inflammation of the brain. (encephalon- brain, *itis*- inflammation). The JE virus causes inflammation of the nerve cells (encephalitis) or the surrounding membranes (meningitis)[1]. National Vector Born Disease Control Program[2] reported 4511 confirmed cases of JE and 788 Deaths because of JE in India, from 2008 till 31st October 2013.

Japanese encephalitis can be prevented by a vaccine. More than 88 million children have been administered sa-14-14-2 in India. Treatment for Japanese encephalitis is supportive; with assistance given for feeding, breathing or seizure control as required, there is no specific treatment for Japanese encephalitis. About 30% of cases showed encephalitis with reduced level of consciousness. It affects legs more than the arms[3,4]. Therefore there is an urgent need for screening new compounds against JEV.

The Envelope protein of the flaviviruses plays a role in viral attachment, fusion, penetration,

hemagglutination, host range and cell tropism, and virus virulence and attenuation[5]. Domain III (DIII) is the major antigenic domain of the envelope protein[6], out of the three structural domains of E protein. Various vaccine strategies being developed are targeted at D III protein region of JEV. Lutein is known as a carotenoid vitamin. Lutein has previously shown inhibitory activity against the hepatitis B virus (HBV) full-length promoter (Fp). The study suggests that lutein possesses an anti-HBV activity and exerts its antivirus effects by inhibiting transcription[7-10] of HBV. Rosmarinic Acid (RA) is a potent antiviral agent against JE. Various in-vivo experiments have clearly indicated that RA reduces the viral replication within the brain[11]. The ligands of lutein and rosamarinic acid were selected on the basis of their structure based Virtual Screening from ZINC database. Virtual screening or docking makes it feasible to screen thousands of compounds against one or more protein targets in less time. Small molecule databases, in public domain such as ZINC, Pubchem, ChemDB, Chem Spider, KEGG ligand database and Drug Bank are available for virtual screening.

MATERIALS AND METHODS

preparation of receptor:

The structures of domain III protein of Japanese encephalitis virus, which was resolved using triple-resonance NMR spectroscopy[6] were downloaded from RCSB Server Protein Data Bank (PDB ID: 1PJW). Structural anomalies in the side chain and folds were corrected and the structure was energy minimized using SPDBV tool. The modified structure so obtained was saved in *.pdb* format and used for all further docking studies.

preparation of ligands:

The zinc database was searched for ligands of Lutein, Rosamarinic acid and Zinc08221225, Zinc14879959, Zinc14879961, Zinc40164432, Zinc 00901160, Zinc 899870 were retrieved ligands were screened with ADME constraints according to Lipinski's rule[12]. The selected ligands were prepared using the Marvin Sketch software V.5.10 [13], Addition of missing hydrogen atoms and fidelity of all bonds were checked using "add hydrogens" and "Clean Hybridization" options respectively.

Structure of various ligands taken from ZINC database were docked *in silico* with protein domain III using iGEMDOCK software V.2.1[14-18].

prediction of pharmacological property:

Lipinski Filter was utilized to screen the ligand, based on Lipinski's Rule of Five and Oral Bioavailability. The parameters of the Lipinski's rule are as follows: the molecular weight must be < 500 Da, Log P < 5, the number of hydrogen donors must be < 5, the number of acceptor hydrogen's must be < 10, and the refractivity molar range must be between 40–130. Molecular descriptors, such as Log P, the number of hydrogen bond donors, the number of hydrogen bond acceptors, and the molecular mass of the compounds were analyzed by utilizing Lipinski Filter (Table 1).

prediction of toxicity :

Lazar a software package is used to detect mutagenic and/or carcinogenic properties based on the similarities in functional group with mutagenic and/or carcinogenic ones present in its database (Table 2)[19].

RESULTS AND DISCUSSION

The rosamarinic acid showed activity *in vivo* as mentioned earlier in the paper. According to the work carried out in our laboratory, lutein also showed potential anti-jev activity (unpublished data.)The docking of protein domain III with ligands using iGEMDOCK, shows arrangement of ligands based on increasing order of energy. The analyzed results suggest that Rosamarinic acid with ligand Zinc 00901160 (-126 KCal/Mol) has lesser energy than the others (Table 3). Further study of the entire docked structure showed that H-bond and weak van der Waals interactions were formed between ligand and receptor, especially with amino acids lysine, tyrosine, proline, alanine and phenylalanine. Although the ligand Zinc 00901160 satisfies all the Lipinski rule of 5 but it also non

carcinogenic, as stated in Lazar server. For further verification of our study, the Zinc ligand 00901160 should be studied *in vitro*, with some modification. However, as this present work is only a step forward towards understanding the mechanistic insights of a potent ligand for Japanese Encephalitis Virus (JEV) envelope protein domain III, further *in vitro* and *in vivo* validations are required.

REFERENCES

1. Gruber P ., A. Rubinstein A., Li, P. Bass V.H.K., Robinson J.R., Gastric emptying of nondigestible solids in the fasted dog, J. Pharm. Sci. 1987; 76 : 117–122.
2. Zoonosis division,NICD/ WHO India. Guidelines for Prevention and Control of Japanese Encephalitis: NATIONAL INSTITUTE OF COMMUNICABLE DISEASES 2006. NICD, 2006.
3. Directorate of National Vector Borne Disease Control Programme- Delhi Details of AES/JE Cases and Deaths from 2008-2013. http://www.nvbdcp.gov.in/Doc/JE-cases-death-Oct13.pdf. accessed [on 4/11/13]
4. Ghoshal A., Das S., Ghosh S., Mishra M.K., Sharma V., Koli P. et al., Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis, Glia 2007; 55(5) : 483-96.
5. King N.J., Getts D.R., Getts M.T., Rana S., Shrestha B. et al., Immunopathology of flavivirus infections, Immunology and cell biology 2007; 85(1) : 33-42.
6. Fields B.N., Knipe D.M., Howley P.M., Griffin D.E. Fields virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. xix, 3087 p. p.
7. Wu K.P., Wu C.W., Tsao Y.P., Kuo T.W., Lou Y.C., Lin C.W. et al., Structural basis of a flavivirus recognized by its neutralizing antibody: solution structure of the domain III of the Japanese encephalitis virus envelope protein, The Journal of biological chemistry 2003; 278(46) : 46007-46013.
8. Brown L., Rimm E.B., Seddon J.M., Giovannucci E.L., Chasan-Taber L., Spiegelman D. et al., A prospective study of carotenoid intake and risk of cataract extraction in US men, The American journal of clinical nutrition 1999; 70(4) : 517-524.
9. Chasan-Taber L., Willett W.C., Seddon J.M., Stampfer M.J., Rosner B., Colditz G.A.et al., A prospective study of carotenoid and vitamin A intakes and risk of cataract extraction in US women. The American journal of clinical nutrition 1999; 70(4) : 509-516.
10. Cho E., Seddon J.M., Rosner B., Willett W.C., Hankinson S.E., Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. Archives of ophthalmology 2004; 122(6):883-892.
11. Pang R., Tao J.Y., Zhang S.L., Zhao L., Yue X., Wang Y.F.et al., In vitro antiviral activity of lutein against hepatitis B virus, Phytotherapy research : PTR 2010; 24(11):1627-16230.
12. Swarup V., Das S., Ghosh S., Basu A., Tumor necrosis factor receptor-1-induced neuronal death by TRADD contributes to the pathogenesis of Japanese encephalitis, Journal of neurochemistry 2007;103(2):771-783.
13. http://www.scfbio-iitd.res.in/utility/LipinskiFilters. [accessed on 4/11/13]updated 2008; cited [2013 4/11].
14. http://www.chemaxon.com/download/marvin. [accessed on 4/11/13]
15. Yang J.M., Chen C.C., GEMDOCK: a generic evolutionary method for molecular docking. Proteins 2004; 55(2) : 288-304.
16. Yang J.M., Chen Y.F., Shen T.W., Kristal B.S., Hsu D.F., Consensus scoring criteria for improving enrichment in virtual screening, Journal of chemical information and modeling 2005; 45(4) : 1134-1146.
17. Yang J.M., Kao C.Y., Flexible ligand docking using a robust evolutionary

algorithm, Journal of Computational Chemistry 2000; 21(11) : 988-998.
18. Yang J.M., Shen T.W., A pharmacophore-based evolutionary approach for screening selective estrogen receptor modulators, Proteins 2005; 59(2) : 205-220.
19. Yang K.D., Yeh W.T., Chen R.F., Chuon H.L., Tsai H.P., Yao C.W. et al., A model to study neurotropism and persistency of Japanese encephalitis virus infection in human neuroblastoma cells and leukocytes, The Journal of general virology 2004; 85 : 635-642.
20. GMBH Insilico toxicity. gmbh; [Acessed 2013 6/11]; Available from: http://lazar.in-silico.de/predict. accessed on

TABLES:

Table 1: Summary of Lipinksy Filter Analysis of Ligands

Ligand	Mol. Wt.	H-bond Acceptor	H-bond donor	*LogP*	Molar Refractivity
Zinc 08221225	568	*0*	*2*	10.414	184.144
Zinc14879959	568	*0*	*2*	10.414	184.144
Zinc 14879961	568	*0*	*2*	10.414	184.144
Zinc 40164432	568	*0*	*2*	10.414	184.144
Zinc 00899870	359	4	4	1.639	87.873
Zinc 00901160	359	4	4	1.639	87.873

It can be predicted that lutein ligands (Zinc08221225, Zinc 14879959, Zinc 14879961, Zinc 40164432) were not following few of the parameter of the Lipinski filter rule such as molecular weight must be < 500 Da, Log P < 5, refractivity molar range must be between 40–130. Where as Rosamarinic acid ligands (Zinc 00899870 and Zinc 00901160) have cleared Lipinski filter .

Table 2: Summary of Lazar Server Result

Ligand	FDA v3b Maximum Recommended Daily Dose mmol:	DSSTox Carcinogenic Potency DBS Mouse:
Zinc 08221225	0.0200397960283797 (**Confidence** : 0.214)	non-carcinogen (**Confidence** : 0.163)
Zinc14879959	0.0200397960283797 (**Confidence** : 0.214)	non-carcinogen (**Confidence** : 0.163)
Zinc 14879961	0.0200397960283797 (**Confidence** : 0.214)	non-carcinogen (**Confidence** : 0.163)
Zinc 40164432	0.0200397960283797 (**Confidence** : 0.214)	non-carcinogen (**Confidence** : 0.163)
Zinc 00899870	0.0129355580197732 (**Confidence** : 0.109)	non-carcinogen (**Confidence** : 0.257)
Zinc 00901160	0.0129355580197732 (**Confidence** : 0.109)	non-carcinogen (**Confidence** : 0.0136)

It can be predicted that all the ligands of lutein and rosamarinic acid showed non carcinogenic property.

Table 3: Fitness Table of Lutein and Rosamarinic Acid

Compound	Energy	VDW	HBond	Elec
1PJWEM-zinc_40164432	-129.91	-124.457	-5.45309	0
1PJWEM- zinc_00901160	-126.569	-81.4895	-43.3208	-1.75873
1PJWEM-zinc_14879959	-126.31	-119.868	-6.44165	0
1PJWEM-zinc_82212251	-119.95	-119.95	0	0

1PJWEM-zinc_14879961	-119.347	-117.649	-1.69797	0
1PJWEM-zinc_ 00899870	-119.178	-73.1948	-44.0736	-1.90965

It can predicted that the though Zinc ligand 40164432 was showing good binding energy but was not following lipinsky filter whereas ligand Zinc 00901160 (Rosamarinic acid) showed energetically favorable interaction in terms of binding energy and Vander wall's interaction and electrostatic and hydrogen bonding.

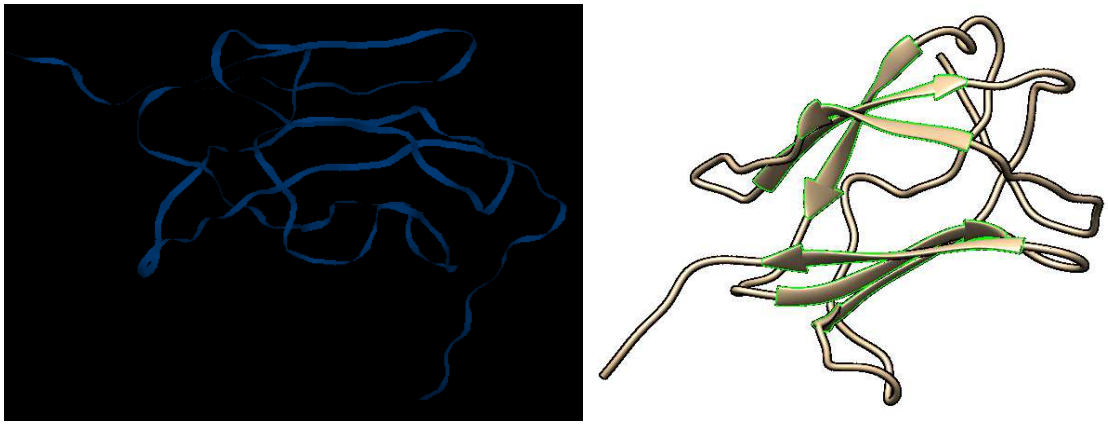

Fig. 1:Screenshot of receptor shown alone in UCSF Chimera before docking

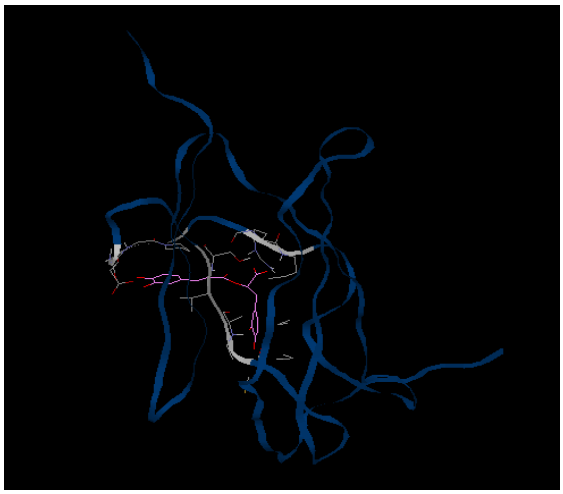

Fig.2: Screenshot of receptor with Ligand Zinc 00901160 in UCSF Chimera