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Research Article

THERAPEUTIC POTENTIAL OF IMMUNOSUPPRESSANT DRUGS AGAINST SPONDWENI VIRUS INFECTION

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ABSTRACT

Spondweni virus infects humans to cause febrile illness which is similar to Zika virus infection. Both viruses have almost similar NS5 protein which interacts with host cyclophilin protein to cause the disease. Cyclophilin is a member of cellular peptidyl-prolylisomerase (PPIase) family, which plays a role in flavivirus replication. The immunosuppressant drug cyclosporine inhibits the flavivirus replication by blocking the interaction between host cyclophilin and viral NS5 protein. As no specific treatment is available to prevent Spondweni virus infection, we have investigated the *in silico* therapeutic potential of two selected immunosuppressant drugs cyclosporine A and isogarcinol (CID 5284373; 11135781) against Spondweni infection using molecular docking analysis. The 3D model of Spondweni virus NS5 protein region (YP_009222008.1:2532-3413) was predicted using Zika virus NS5 model (PDB ID: 5TFR with query coverage 99% and identity 78%) as template. Further *in silico* docking interaction was performed to investigate the residues involved in interaction. The interaction of cyclosporine A and isogarcinol with the target protein of Spondweni virus suggested that both immunosuppressants interacted with prominent binding site with good docking score and might be involved in blocking the binding of host cyclophilin to Spondweni virus protein. Thus both immunosuppressants cyclosporine A and isogarcinol can be explored as a promising drug for the prevention of Spondweni virus infection. When both immunosuppressants are compared, cyclosporine A might act better than isogarcinol and have greater therapeutic potential against Spondweni virus disease.

INTRODUCTION

Spondweni virus, a member of the family Flaviviride and genus Flavivirus, is spread by Aedes mosquitoes, such as *A. aegypti* and *A. albopictus*, and *Culexquinque fasciatus* which are active during day time. It was reported primarily in monkeys and later in humans. Recently, it has been reported from more than 20 countries. In addition to local transmission, Spondweni virus infections also spread worldwide by international travelling (Camila *et al*, 2015). Spondweni virus disease is related to Zika infection, yellow fever, Japanese encephalitis and west Nile virus disease (Andrew *et al*, 2016).The most common symptom of this virus is fever, rash, joint pain, conjunctivitis, muscle pain and headache, epistaxis, vertigo, photophobia, disorientation, maculopapular and pruritic rash **(**Kaslow *et al***,** 2014). Spondweni virus genome is non segmented, single stranded RNA, approximately 11 kilobases in length which can be directly translated into viral proteins. It encodes seven non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 and three structural proteins capsid protein C, envelope protein E and premembrane/ membrane protein (Martin S *et al*, 1982). The RNA-dependent RNA polymerase NS5 protein of Spondweni virus strain SM-6 V-1 is about 70% similar to the Zika virus and Japanese encephalitis virus NS5 protein which interacts with host cyclophilin protein to cause the disease. Cyclophilin is a family of cellular peptidyl-prolylisomerases (PPIase), which play a role in flavivirus replication. Cyclosporine binds and inhibits the flavivirus replication through blocking the interaction between host cyclophilin and viral NS5 protein. It also binds with HIV1 protein and blocks HIV1 replication in vitro (Min *et al*, 2009).Cyclosporine A and isogarcinol are immunosuppressant drugs, widely used in organ transplantation to prevent rejection (Calne *et al*,1979; Juren *et al*, 2013). It reduces the activity of the immune system by

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interfering with the activity and growth of T cells. Till date there is no specific treatment for Spondweni disease.

Based on the above information, the *in silico* therapeutic strategy was developed by comparative modelling to construct the structure of Spondweni NS5protein region using Zika virus protein model as a template. Further its docking calculation was performed with immunosuppressants cyclosporine A and isogarcinol to see their binding and selection of better immunosuppressant.

MATERIALS AND METHODS

Retrieval of the sequence of Spondweni virus protein and search of its homologs in Zika virus and phylogeny

The Spondweni virus strain SM-6 V-1 protein sequence (YP_009222008.1:2532-3413) was retrieved from NCBI database (Li *et al*, 2017). The NS5 conserved sequence was searched from NCBI-CDD database (Marchler-Bauer *et al*, 2015).The homology search of retrieved protein sequence was done using BLAST tool against PDB database with selected template model for 3D structure prediction. The multiple sequence alignment of Spondweni virus NS5 protein region was performed using ClustalW tool (Thompson *et al*, 1994) with their closely related organisms (Zika virus, St Louisencephalitis virus, Ilheus virus, West Nile virus). Further phylogenetic tree was constructed using MEGA tool (Kumar *et al*, 1994). The closest homolog structure of the full-length Zika virus NS5 (PDBID: 5TFR) was selected as template using PDB advanced search for commutative modelling (Lensink *et al*, 2017).

Homology modelling of Spondweni virus

The Spondweni virus strain SM-6 V-1 NS5 conserved protein sequence was used for homology modeling using the best identified homologous template structure of Zika virus NS5 region (PDB ID: 5tfr)using Discovery Studio 3.5 (Shahi *et al*, 2013).

Retrieval of the immunosuppressants cyclosporine A and isogarcinol as a ligand

Cyclosporine A and isogarcinol are immunosuppressant drugs. They bind and inhibit the flavivirus replication through blocking the interaction between host cyclophilin and viral NS5 protein. It also binds with HIV1 protein and blocks HIV1 replication in vitro. The PubChem compound database was used for retrieval of immunosuppressant drugs cyclosporine A and isogarcinol. The drug likeness property of cyclosporine A and isogarcinol was checked by Lipinski filter (Lipinski *et al*, 1996).

Molecular docking analysis

Flavivirus NS5 is most conserved amongst the viral proteins. It is about 900 kDa and plays a vital role in virus replication (Lim*et al*, 2015). Its N-terminal domain encodes dual N7 and 2'-O methyl transferase activities (MTase), and guanylyltransferase (GTase) is involved in RNA cap formation. The C-terminal region comprises a RNA-dependent RNA polymerase (RdRp) required for viral RNA synthesis. Both MTase and RdRp activities of dengue virus NS5 are well characterized, structurally and functionally (Liu *et al*, 2017). The docking calculation was done with Patchdock server. Further docking experiment was performed between immunosuppressant drugs cyclosporine A and isogarcinol and Spondweni Virus NS5 protein model as receptor. The docking assessment was done using Discovery Studio 3.0to find out the contacting receptor residues involved in interaction with cyclosporine A and isogarcinol with their docking score (Singh *et al*, 2017; Tayebeh *et al*, 2017).

RESULTS AND DISCUSSION

Homologs of Spondweni Virus NS5 protein and their phylogeny

The sequence of Spondweni virus NS5 region YP_009222008.1:2532-3413) was retrieved for phylogeny classification. The homologous protein sequences from closely related organisms were retrieved based on maximum 100% query coverage with 70% identity parameter. The four viral NS5 protein regions from Zika virus (AY632535.2), St Louisencephalitis virus (YP_001008348.1), Ilheus virus (YP_001040006.1) and West Nile virus (YP_001527877.1) were used for multiple sequence alignment and phylogenetic classification. Then the homologous sequences were used for multiple sequence alignment with the help of clustalW and phylogenetic classification by MEGA tool. The results elucidate that Spondweni virus is closely similar with Zika virus (Fig.1a).

All homologous structure sequences were also retrieved for Japanese Encephalitis virus, West Nile virus and Zika virus for phylogeny study with Spondweni virus. Only three suitable PDB structures were retrieved (PDB IDs: 2HFZ, 4K6M and 5TFR) for phylogenetic classification. The sequential classification also revealed that Zika virus NS5 protein region was closely similar with Spondweni virus. Further structural phylogeny was also constructed on the structural basis using SALIGN (Braberg *et al*, 2012). The phylogenetic based structural classification using SALIGN revealed that Zika virus NS5 protein region was found closely related with Spondweni virus (Fig. 1b). Then on the basis of phylogenetic based structural classification, structure of Zika virus NS5 protein region (PDBID: 5TFR) was selected for homology modeling.

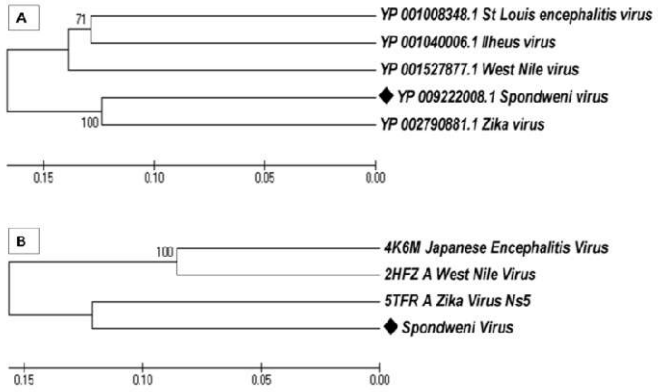

Figure 1 Phylogenetic classification **(A)** on the basis of sequence similarity, **(B)** on the basis of structure similarity

Homology modeling of Spondweni virus NS5 protein region and submission of predicted model

The Spondweni virus NS5 protein model was successfully generated with the help of selected suitable template of Zika virus NS5 (PDB ID: 5TFR). The VADAR statistics of

predicted model has 43% helices, 21% strands and 34% loops. The core size of the predicted model was 78.1%. The RAMPAGE statistics of predicted model having 98.4% amino acids were in favoured regions which were close to expected value and 1.4% amino acids were in allowed regions, which were also close to expected value, only 0.2% residues fall in disallowed region. Similarly, PDBSum server results predicted that over 90% of the residues in the most favoured region indicated good quality of predicted model. Further predicted reliable model was deposited in PMDB database with PMDB ID: PM0080779 (Fig. 2, 3).

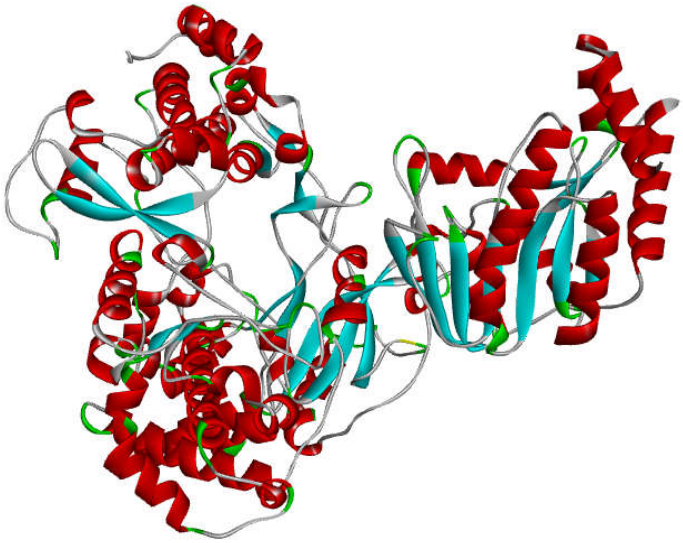

Figure 2 Predicted model of Spondweni virus NS5 Protein

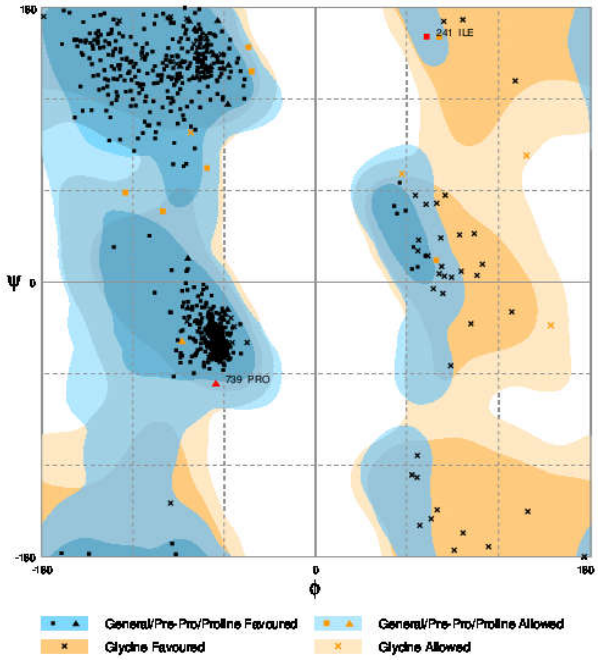

Figure 3 Ramachandran plot for predicted model

Retrieval of the immunosuppressants cyclosporine A and isogarcinol (CID 5284373; 11135781) as ligands

Retrieval of the immunosppressants cyclosporine A PubChem CID: CID 5284373 and Isogarcinol PubChem CID: 11135781was done using PubChem compound database. The druglikeness properties of selected immunosuppressants was calculated using Lipinski filter (Farhadi *et al*, 2017). Results of Lipinski filter for immunosuppressants following 2 drug likeness properties are presented in Table 1. The Lipinski Rule of five is a thumb rule to evaluate drug likeness or determine if a chemical compound with a pharmacological or biological activity has properties that would make it an orally active drug in humans. Lipinski rule of 5 helps in distinguishing between drug like and non-drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules.

Table 1 Drug likeness property of immunosuppressants Cyclosporine A and Isogarcinol

Name of compound	Molecular weight	H- bond donor	H- bond acceptor	Log P	Refractive Index
Standard	>500	>5	>5	>10	40-130
Cyclosporine A	1201	5	20	2.26	327.2
Isogarcinol	602	2	6	8.58	173.3

Interaction of Spondweni virus NS5 Protein model with (a) cyclosporine A and (b) isogarcinol

Patchdock server was used for docking calculation. Before performing docking docking analysis, active site prediction was done using Discovery studio 3.5. In Patchdock docking calculation, both immunosuppressants Cyclosporine A and Isogarcinol interacted with prominent site of protein receptor identified using discovery studio 3.5 with docking score 10566 and 6298 respectively (Table 2; fig. 4-6). The receptor interacting residues were involved in interaction with Cyclosporine A and Isogarcinol, which belong to major active site of the Spondweni virus NS5 protein model Table 3.

Table 2 Interaction of immunosuppressants with Spondiweni virus NS5 region protein

Name of interacting compound	Score	Area
Cyclosporine A	10566	1408
Isogarcinol	6298	716.0

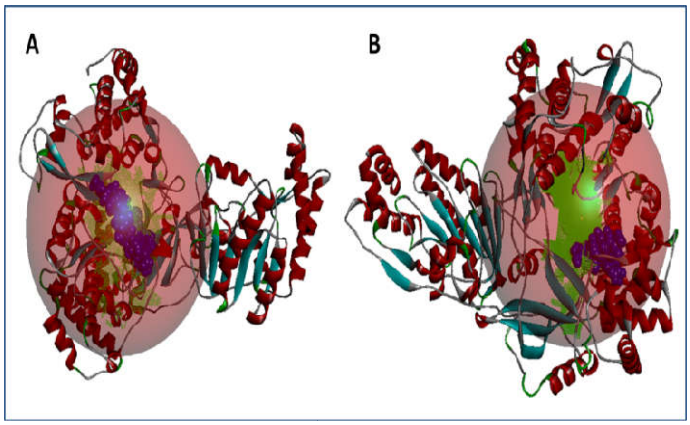

Figure 4 Interaction of immunosuppressants with Spondweni virus NS5 protein. (A) Cyclosporin A, (B) Isogarcinol.

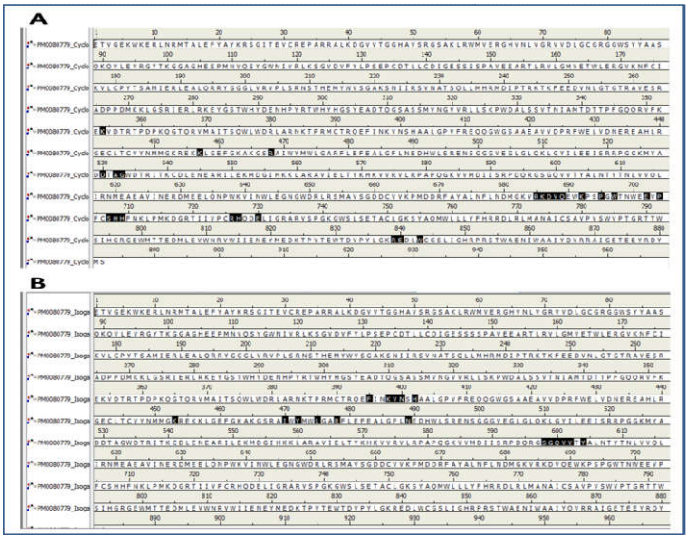

Figure 5 Amino acids sequence that interact with immunosuppressants .(A) cyclosporine A, (B) Isogarcinol.

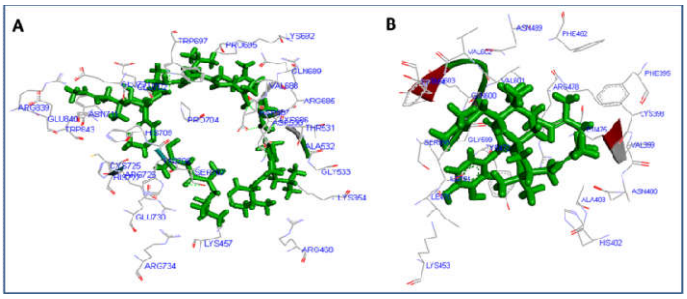

Figure 6 A 2-D structure with interacting residue of immunosuppressants. (A) cyclosporine A, (B) Isogarcinol.

Table 3 The residues of Spondweni virus NS5 protein involved in interaction with selected immunosuppressants.

Name of Compounds	Interacting residues
Cyclosporine A	Lys^{354}, Lys^{457}, Asp^{530}, Ala^{532}, Gly^{533}, Arg^{685}, Lys^{686}, Asp^{687}, Val^{688}, Gln689, Lys^{692}, Pro^{695}, Trp^{697}, Glu^{702}, Pro^{704}, Ser^{707}, His^{708}, His^{709}, Arg^{726}, His^{727}, Glu^{730}, Arg^{839}, Glu^{840}, Trp^{843}
Isogarcinol	Phe^{395}, Lys^{398}, Val^{399}, Asn^{400}, His^{402}, Lys^{453}, Ile^{470}, Tyr^{472}, Leu^{475}, Arg^{478}, Asn^{489}, Ser^{598}, Gly^{599}, Gln^{600}, Val^{601}, Val^{602}, Tyr^{604}

CONCLUSION

The prominent active site of Spondweni virus NS5 target protein was involved in interaction with cyclosporine A and Isogarcinol immunosuppressants. Interaction visualization also revealed that Spondweni virus NS5 target interacted best with both cyclosporine A and Isogarcinol, which might be inhibit the Spondweni virus NS5 target protein. Thus, the selected immunosuppressants have strong therapeutic potential against Spondweni disease and can be used as drug for the prevention of Spondweni virus associated infections. In comparison to both isogarcinol, cyclosporine A can have greater therapeutic potential against disease associated with Spondweni virus and can be used as a drug for the prevention of infection/disease caused by Spondweni virus.

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Conflict of interests

Authors have no conflict of interest affiliated with this article.

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