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MOLECULAR DOCKING ANALYSIS OF HUMAN TRANSFERRIN AND LACTOFERRIN BINDING PROTEINS OF NEISSERIA MENINGITIDIS WITH 5 HYDROXY 1-4 NAPHTHOQINONE.

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ABSTRACT

Neisseria meningitidis is the principal cause of bacterial meningitis.. Bacteria can be transmitted by carriers through the droplets of respiratory secretions and can only infect humans. The mortality rate of bacterial meningitis is very high even after the discovery of many antibiotics. Acquisition of iron is the major determinant in the pathogenesis of Neisseria meningitidis which is fulfilled by human host proteins like transferrin, lactoferrin, hemoglobin, and haptoglobin-hemoglobin. Present study is performed on transferrin binding protein A, transferrin binding protein B, lactoferrin binding protein A and lactoferrin binding protein B which together constitutes receptors for human transferrin, and lactoferrin iron complex in Neisseria menningitidis which further are responsible for the transport of essential iron. Crystal structures of TbpA and TbpB are taken from Protein data bank while sequences of LbpA and LbpB are retrieved from NCBI and modeled by using Geno3D. Molecular docking studies are performed by taking Juglone or 5 hydroxy 1-4 naphthoqinone as a ligand. Binding energies are calculated and interactions are studied. Our findings suggest Juglone shows effective binding with these proteins with much lesser binding energies can be used for clinical trials for the treatment of meningitis.

KEY WORDS : Transferrin binding protein A (TbpA), Transferrin binding protein B (TbpB), Lactoferrin binding protein A(LbpA), Lactoferrin binding protein B (LbpB)

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INTRODUCTION

N.meningitidis causes three major diseases nasopharyngitis,meningococcal septicemia, and meningococcal meningitis. Nasopharyngitis is usually a short term illness without any prominent symptoms. It is followed by Meningococcal Septicemia in which bacteria colonize in the nasopharynx and spread into the blood stream with the symptoms such as high fever, skin rashes and arthritis. The most common disease caused by Neisseria meningitidis is Meningococcal Meningitis. The disease comes with the symptoms like severe headache, high fever, pain and stiffness of the neck followed by nausea. Disease diagnosis is done by clinical examination followed by a lumbar puncture showing a purulent spinal fluid. There are twelve serogroups of N.Meningitidis out of which six serogroups are (A, B, C, W135, X and Y) disease causing. Oily chloramphenicol or ceftriaxone and vaccines like meningococcal A conjugate vaccine, C conjugate vaccines, tetravalent A, C, Y and W135 conjugate vaccines and meningococcal polysaccharide vaccines are used for the treatment and the control. In order to survive inside the host, bacteria have to scavenge iron and other essential nutrients from the host. Iron uptake is the main cause for virulence in N. meningitidis was first proposed by Payne and Finkelstein (1). N. meningitidis's virulence can be enhanced in experimental infections by injecting iron compounds in animal host (2). Iron deprivation has detrimental effects on the growth of N. meningitidis (3) Intracellular iron in the body is stored in ferritin and hemoglobin (4) while extracellular iron is found attached to high-affinity iron-binding proteins, such as transferrin in serum and lymph and lactoferrin in milk and secretions. The major source of iron available to the meningococci, therefore, is iron complexed to host iron-binding proteins and almost zero free iron for bacterial growth but the organism is capable of acquiring iron under iron-restricted conditions. It has iron acquisition systems which comprises high-affinity receptors for iron-bound host proteins, including transferrin, lactoferrin, and hemoglobin enable it to use these proteins as iron source.(5,6,7,8) Iron dextran or human ferritransferrin when injected into mice with intraperitoneal inoculation of live N. meningitidis resulted in lethal infection, whereas mice injected with only N. meningitidis had transient bacteremia and recovered quickly. (9, 10) Meningococcal infection in mice and in humans can be controlled by decreasing the amount of free transferrin (11). An outer membrane protein called FetA or FrpB is identified in N. gonorrhoeae, has demonstrated low binding affinity and transport of ferric enterobactin (1213,14,15). A homologous protein, with 91% sequence identity has been identified in N. meningitidis which probably has similar functions. (1516). Two more proteins, transferrin-binding protein A (TbpA) and transferrin-binding protein B (TbpB), are also identified in N. meningitidis which together functions as the transferrin receptor (17, 18,19). Mutants lacking both the proteins are unable to utilize iron from transferrin (20). Lactoferrin receptor is also believed to be an important virulence factor of the meningococci. N. meningitidis enters into the body from nasopharynx, where lactoferrin predominates as the main source of iron it then crosses the blood-brain barrier and may also serve as a source of iron during the invasive phase of infection (21,22). The lactoferrin receptor of N. meningitidis, like the transferrin receptor, consists of two protein components, LbpA and LbpB. Two proteins which are responsible for the recognition and transport of heme proteins have also been identified in N.meningitidis which may cause human disease (23).Two proteins of RTX family Frp A and Frp C are also secreted by the organism. Furthermore, high titers of both immunoglobulin G (IgG) and IgA that recognized FrpC were found in the convalescent-phase sera of patients with meningococcal disease (24).In the present study TbpA and TbpB (transferrin binding receptor), responsible for the transport of iron transferrin complex are docked separately with Juglone and LbpA and LbpB (receptor for lactoferrin iron complex) are modeled and modeled structures are docked with the same ligand separately. Juglone which is a

phytonutrient present in walnuts also called 5-hydroxy-1,4-naphthalenedione is an organic compound with the molecular formula $C_{10}H_6O_3$, can interact with some of the major proteins of iron acquisition system of N. meningitidis thus can be used for clinical trials for disease prevention and treatment.

MATERIALS AND METHODS

Computer– Aided Drug Design is a specialized discipline that uses computational methods to simulate drug – receptor interactions and are dependent on bioinformatics tools, applications and databases. (25, 26) The NCBI Entrez protein database which has protein sequences from different sources, that includes Swiss-Prot, protein information resource, protein data bank, Protein research foundation are accessed at internet. Structures of the proteins which form receptor for human transferrin, Transferrin binding protein A (Tbp A) and Transferrin binding protein B (Tbp B) with PDB IDs -3V8X and 3V8U, both from serogroup B are retrieved from PDB which is a repository for the 3-D protein structural data. These structures are obtained by X-ray crystallography or NMR spectroscopy, submitted by Scientists around the world can be accessed at internet. Sequences of Lactoferrin binding protein A and lactoferrin binding protein B precursor were retrieved from NCBI with Accession NOs YP-002343032-1 and AAC38586. Structures are modeled using Geno3D, which is an automated protein modeling web server supplementary to the CATH database. Five models of lactoferrin binding protein A and B have been generated by Geno3d by selecting the protein templates with more than 35 % similarities out of which, models with the lowest energies were used for docking.Structure of ligand which is Juglone (5 hydroxy 1-4 naphthoquinone) with Pubchem ID- CID 3806 is retieved from pubchem and prepared for docking by using OpenBabel.To view protein ligand interactions, to separate crystallized ligands and water molecules from protein and to prepare proteins (Tbp A, Tbp B, Lbp A and Lbp B) for docking, Discovery studio 3.5 from Accelrys is used in the present study, which is a well-known suite of software forsimulating macromolecule syste m, used for development of new drugs, therapeutic antibodies, vaccines,andsynthetic e nzymes.Tbp A and Tbp B were docked with juglone by using Autodock 4, which is simulation software for molecular modeling and Protein-ligand docking available under the GNU General Public License. and frequently used for calculating and displaying feasible docking modes of pairs of protein and corresponding ligands (protein-ligand docking) and for calculating binding energies of their interactions.It has of two main programs:- AutoDock for docking of the ligand to a set of grids describing the target protein and AutoGrid for pre-calculating these grids, maintained by The Scripps Research Institute and Olson Laboratory.Entire surface of the macromolecule is searched for docking. Very large grid maps are created by autogrid covering the entire macromolecule.Docked structures are analyzed by using Discovery studio 3.5 and Pymol 1-1.

RESULTS

Following are the results of molecular docking of proteins of iron acquisition system of N.meningitidis with Juglone (5 hydroxy 1-4 naphthoquinone) which is a phytonutrient present in walnuts.Table 1 and 2 show binding and intermolecular energies of transferrin and lactoferrin binding proteins with juglone respectively while figure 1 and 2 show interactions of residues of transferrin and lactoferrin binding proteins and ligand. Out of 10 docked conformations the best one with minimum binding energy is shown in the following tables and figures. Every conformation is a combination of translation, quaternion and torsion angles and is characterized by intermolecular energy, internal energy and torsional energy. The combination of first and third gives binding energy. Five models of lactoferrin binding protein A and B both have been generated by Geno3d which is an automated protein modeling web server, by selecting protein templates with more than 35 % similarities out of which, models with the lowest model energies of -6659.78 and-16051.00 kcal/mol, were used for docking.Our results

indicate that Juglone shows effective binding with three major iron transport proteins of N. meningitidis namely TbpA,TbpB and LbpA with binding energies of -5.12 kcal/mol, -4.56 kcal/mol and 4.25 kcal/mol respectively, while does not show effective binding with LbpB with binding energy of 0.8 kcal/mol. It has been proved earlier that the fatty acids present in walnuts are also biologically relevant for mental health while our study focuses on juglone and we propose that it can be used for clinical trials for the prevention and treatment of bacterial meningitis otherwise too it would be interesting because it may interfere with iron absorption in human beings.

Table 1

Results of molecular docking of the TbpA and TbpB with Juglone using Autodock4 suite

RECEPTOR	PDB-ID	LIGAND	BINDING ENERGY(kcal/mol)	INTERMOLECULAR ENERGY
Tbp A	3V8X	Juglone	-5.12	-5.42
Tbp B	3V8U	Juglone	-4.56	-4.86

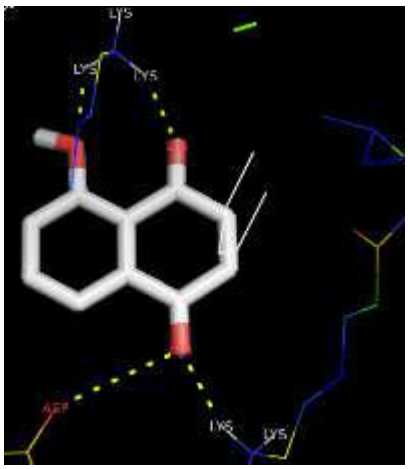
Table 2

Results of molecular docking of the LbpA and LbpB with Juglone using Autodock4 suite

RECEPTOR	MODEL ENERGY(kcal/mol)	ACCESSION NO.	LIGAND	BINDING ENERGY(kcal/mol)	INTERMOLECULAR ENERGY
Lbp A	-6659.78	YP-002343032-1	Juglone	-4.25	-4.55
Lbp B	-16051.00	AAC38586	Juglone	-0.8 (doesn't show effective binding)	-1.1

Figure 1

(a)-Interaction between Transferrin binding protein A of N meningitidis and Juglone
(b) Interaction between Transferrin binding protein B of N meningitidis and Juglone



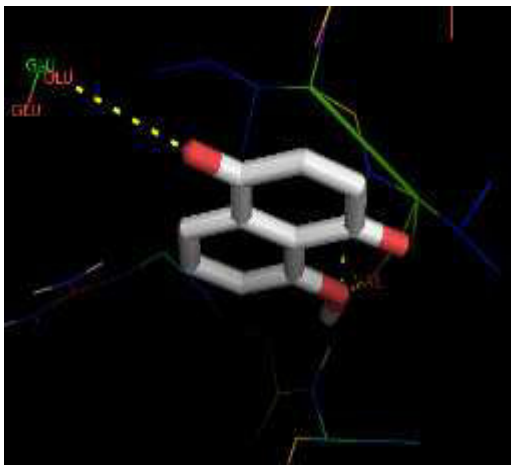
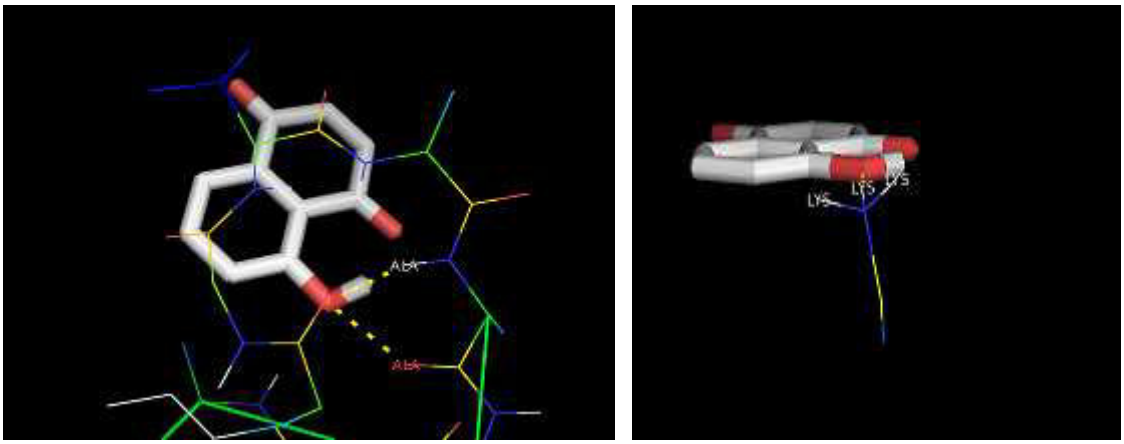


Figure 2
(a)-Interaction between lactoferrin binding protein A of N meningitidis and Juglone
(b) Interaction between lactoferrin binding protein B of N meningitidis and Juglone



DISCUSSION

Walnuts a rich source of unsaturated fatty acids and antioxidants are known to have several medicinal properties.(27) Aged rats fed diets containing 2% to 6% walnuts showed reversal of age-associated motor and cognitive function, but a 9% walnut diet impaired performance (28) therefore eating walnuts in appropriate quantity can nourish the brain and improve cerebral circulation, There are evidences that support an important role of DHA and omega-3 fatty acids present in walnuts in neurodevelopment (29,30,31). As such walnuts don't have any reported drug interactions but may interfere with the absorption of iron if taken in excessive amount and juglone too exhibits some toxic effects in animals but toxicity has not been reported yet on humans. These side effects of walnut are often observed in individuals who over do the eating. Else, if taken in adequate quantity it is healthy and nutritious. The receptor-ligand interactions play a significant role in molecular docking and drug designing. The receptors for human transferrin and lactoferrin iron complex of N. meningitidis interact effectively with the ligand which in the present study is Juglone .On the basis of present study we propose that Juglone can be better used for the development of new therapeutics and for the treatment of meningitis and eating walnuts can lower the risk of meningitis.To the best of our knowledge, no docking experiments have been conducted so far with the constituents of walnut and proteins of N.meningitidis thus we don't have data to compare our study. However, above discussion clearly proves that walnuts as such have many health benefits and medicinal properties many of which are related to central nervous system Therefore, walnuts and it's phytonutrients should be considered for clinical trials in patients with meningitis alongwith the established antibiotic therapies and also in patients at risk for acquiring such infections e,g. immunosuppressed patients, so that more evidence could be obtained.Further this would be interesting to know that juglone which is showing interactions with iron transport proteins of N. meningitidis, may interfere with iron transport in human beings.

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