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Homology modelling and molecular docking studies of human placental cadherin protein for its role in teratogenic effects of anti-epileptic drugs

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ABSTRACT

Anti-epileptic drugs (AEDs) have high risk of teratogenic side effects, including neural tube defects while mother is on AEDs for her own prevention of convulsions during pregnancy. The present study investigated the interaction of major marketed AEDs and human placental (hp)-cadherin protein, *in-silico*, to establish the role of hp-cadherin protein in teratogenicity and also to evaluate the importance of Ca^{2+} ion in functioning of the protein. A set of 21 major marketed AEDs were selected for the study and 3D-structure of hp-cadherin was constructed using homology modelling and energy minimized using MD simulations. Molecular docking studies were carried out using selected AEDs as ligand with hp-cadherin (free and bound Ca^{2+} ion) to study the behavioural changes in hp-cadherin due to presence of Ca^{2+} ion. The study reflected that four AEDs (Gabapentin, Pregabalin, Remacimide and Vigabatrine) had very high affinity towards hp-cadherin and thus the later may have prominent role in the teratogenic effects of these AEDs. From docking simulation analysis it was observed that Ca^{2+} ion is required to make hp-cadherin energetically favourable and sterically functional.

1. Introduction

Epilepsy is the malfunctioning of the brain that disturbs the normal brain electrical activity between nerve cells and generate seizures (Jefferys, 2010; Scharfman, 2007). A list of structurally diverse anti-epileptic drugs (AEDs) is presently used for clinical therapeutic application for this disorder (Stafstrom, 2010). Despite the wide variety of available treatments, approximately 30% of people with epilepsy fail to respond satisfactorily to first line anti-epileptic drugs (Remy and Beck, 2006). There is, therefore, an important unmet clinical need for new antiepileptic therapeutics with more specific mechanisms of action, fewer side effects and increased potency. However, the clinical use of AEDs are associated with one life threatening side effect *i.e.*, teratogenicity (Tomson and Battino, 2009). Epileptic pregnant women with AEDs medication may incur the possibility of fetal abnormalities. On the other side, discontinuation of medication may lead to the uncontrolled condition for both mother and child. Thus the risk associated with uncontrolled seizures has to be balanced against the teratogenic risks imposed by the AEDs (Eroglu et al., 2008). Crucial Developmental process from fertilization till the end of gastrulation phase can be altered and may result in structural abnormalities. Multiple congenital anomalies arising from developmental defects are results of fetal exposure to antiepileptic drugs (Hill et al., 2010). They are the leading cause of infant mortality in the first year of life (Martin et al., 2004). The activities of placental proteins may lead to the fetal exposure to AEDs and thus causes teratogenicity (Atkinson et al., 2007). Teratogenicity depends upon the ability of the agent to cross the placenta during rapid differentiation phase (Darsow et al., 2012). Placenta produces a variety of proteins that have specific spatiotemporal attributes. These proteins aid in development of fetus from the blastocyst implantation till the natality (Takahashi et al., 2013). There are various kinds of proteins that bind firmly to the placenta. Placental transport proteins regulate absorption, distribution, and excretion of drugs and thus influence drug disposition, therapeutic efficacy and adverse drug reactions in the human (fetal) body (ITC et al., 2010).

Cadherins are calcium dependent cell–cell adhesion protein with prominent expression in neural tissue. Their extracellular portions is conserved having calcium binding domains that contains 5–34 definitive Ca^{2+} binding domains repeat of ~110

Abbreviations: AEDs, anti-epileptic drugs; AMBER, assisted model building with energy refinement; GUI, graphical user interface; GROMOS, GROningen molecular simulation; VPA, valproic acid; MD, molecular dynamics.

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residues (Angst et al., 2001). The adhesion is mediated by a homophilic mechanism that is cadherin–cadherin binding and the specificity of this binding is determined by the amino terminal homologues repeat segment (Shinoura et al., 1995). The dysfunctioning of cadherins based adhesive system may alter functional connectivity and coherent information processing in the human brain resulting in neurological disease. Several studies have been reported about the upregulation of E-cadherin expression due to interactions AEDs with epigenomic factors (Zhang et al., 2011; Rafal and Henley, 2007; Nam et al., 2004) but there is paucity of information about AEDs interacting directly with cadherin proteins, particularly placental cadherin and thus involved in teratogenicity. Therefore the present study focussed on defining the mode of potential interactions of AEDs with human placental (hp)-cadherin protein, using molecular docking studies, to delineate the AEDs having maximum interaction with hp-cadherin protein. Also, the study involved investigation into the molecular dynamics of hp-cadherin protein to decipher the role of Ca^{2+} ion for its functioning.

2. Materials and methods

2.1. Homology modelling and model evaluation

The 3D-strucutre of hp-cadherin was constructed using homology modelling method, the same being unavailable in Protein Data Bank. For homology modelling, the sequence of hp-cadherin was retrieved from the NCBI protein sequence database and template was identified using PSI-BLAST against the RCSB protein data bank (PDB). The 3D-structure was built using Swiss-Model server in template mode (Arnold et al., 2006). The modelled structure was subjected to validation and assessment using protein structure and model assessment tools at Swiss-Model server using different estimation patterns. The selected model was validated for packaging quality using Anolea plot (Melo and Feytmans, 1998) while Qmean (Benkert et al., 2009) score count was used for local and global analysis of different regions within the model. Gromos MD simulation plot was applied on selected model for analysis of experimentally obtained conformations (Van Gunsteren, 1996) while PROCHECK (Ramachandran plot) was used to analyze the stereochemical and overall quality of the structure (Laskowski et al., 1993). Removal of unfavourable non-bonded contacts was done by energy minimization using GROMOS96 force field in Swiss PDBViewer.

2.2. Molecular dynamics simulation

The best model structure selected from homology modelling was further refined by explicit solvent MD-simulation of hp-cadherin was performed using OpenMM Gromacs (Eastman et al., 2013). The starting structure was solvated in a rectangular box of TIP3P (three-site models have three interaction sites, corresponding to the three atoms of the water molecule) and sodium ions were also added to produce a net neutral system by replacing randomly chosen waters with ions. The missing atoms were constructed using Modeller script of OpenMM Gromacs. The .top and .gro files were generated using GUI interface of OpenMM Zephyr (Friedrichs et al., 2009). Electrostatic interactions were calculated using the Particle Mesh Ewald (PME) formalism. The Langevin dynamics, for constant temperature control, was applied on non-hydrogen atoms at 310 K with friction coefficient of 1 ps^{-1}. The pressure of the system was controlled by Langevin piston at 1.01 bar with an oscillation time of 200 fs and damping time constant of 100 fs. $AMBER_{99}SB$ force–field parameter set was used for simulating the system. The system was then minimized for 10,000 steps and then equilibrated for 10,000 steps. The coordinates and velocities were stored every 25 ps. The simulated and refined structure of hp-cadherin thus obtained was used for further investigation.

2.3. Molecular docking

The structure files (pdb-format) of 21 major marketed AEDs (Supplementary data Fig. S1) were collected from DrugBank (Wishart et al., 2008) and were used for molecular docking studies against modelled hp-cadherin protein using Autodock 4.2.5.1 (Morris et al., 2009). Lamarckian model of genetics were used in which environmental adaptations of an individuals phenotypes are reverse transcribed into its genotype and become heritable traits. Only polar hydrogen was added to the protein and Kollman and Gastegier charges were assigned. The spacing between grid points was set to default value of 0.375 Å. The grid box was set to 480 Å × 260 Å × 280 Å (*x*, *y* and *z* axis) to include all the amino acid residues that were present in protein. The study was focussed primarily on deciphering the binding domain search of each AED and hence blind docking was used for the purpose. The grid size selected for docking was huge to include all the domains of protein. A total of 50 independent runs were performed with a step sizes of 0.2 Å for translations and 5^0 for orientations and torsions. The maximum number of generations was set to 1000 and maximum number of top individuals that automatically survived was set to 1 with mutation rate of 0.02, crossover rate of 0.8, cluster tolerance 0.5 Å, external grid energy 1000.0.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.compbiolchem.2015.11.003.

3. Results and discussions

3.1. Homology modelling and model evaluation

The target sequence of human placental cadherin (CADH3) having 829 amino acid was retrieved from the NCBI protein database with accession no. P22223.2. PDB-1Q55 was identified as template using PSI-BLAST having 54% identity and 98% query coverage. The structure was modelled using Swiss model online server. The structures generated were assessed using Protein Structure and Assessment Tools at Swiss Model server. The selected model (Fig. 1) had the predicted Dfire energy (Zhou and Zhou, 2002) and QMEAN6 score −535.99 and 0.590 $kJmol^{-1}$ respectively.

The DFIRE energy statistical energy provides an accurate loop prediction at a fraction of computing cost required for more complicated physical based energy functions. The predicted Z-score for model was −2.04. The Anolea, Qmeand and Gromos plots (Fig. 2) reflected the quality and acceptability of the modelled structure. The green colour in the plot represented favourable energy whereas red colour represented unfavourable energy

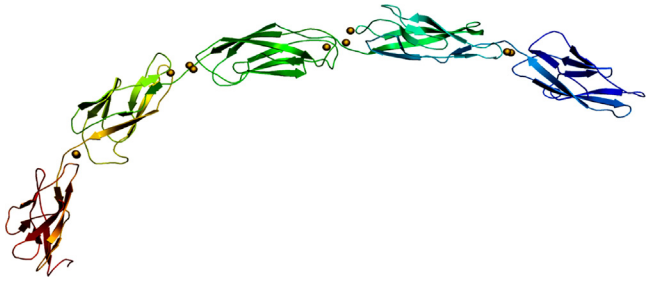

Fig. 1. Homology modelled structure of human placental (hp) cadherin protein.