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Original Article

IN SILICO SCREENING OF PHYTOCHEMICAL COMPOUNDS TARGETING CHILDHOOD ABSENCE EPILEPSY (CAE)

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ABSTRACT

Objective: Childhood absence epilepsy (CAE) is the most frequent pediatric epilepsy syndrome. Since, the mechanism of the disease is not well defined, evidence of successful treatment for CAE is not found till date. Hence, an attempt is made to identify novel plant based compounds which may be effective against Pediatric epilepsy syndrome, to meet this demand for newer drugs with minimal side effects.

Methods: We performed a virtual screening of different synthetic compounds / current medications namely ethosuximide, valproic acid, lamotrigine, carbamazepine and vigabatrin were compared against plant derivatives.

Results: Cannabidivarin is a potent inhibitor against gamma 2 subunit of $GABA_A$ receptors by forming a maximum of number of interactions with the docking score (-5.3).

Conclusion: The plant compound Cannabidivarin (CBDV) may serve as a novel drug with definite control over childhood absence epilepsy.

Keywords: CAE; Mutation; Modeling; Virtual screening; CBDV.

INTRODUCTION

$GABA_A$ receptors are the main mediators of fast inhibitory synaptic transmission within the mammalian central nervous system [1]. They are members of the cys-loop family of ligand-gated ion channels [2]. These transmitter-gated ion channels are assembled as a pentameric complex of subunits which, includes two α subunits, two β subunits, and a γ or δ subunit [3]. $GABA_A$ receptors intercedes both phasic inhibitory synaptic transmission and tonic perisynaptic or extrasynaptic inhibition [4].Genetic epilepsy syndromes including childhood absence epilepsy (CAE), juvenile myoclonic epilepsy (JME), pure febrile seizures (FS), generalized epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome (DS)/severe myoclonic epilepsy in infancy (SMEI) have been associated with different mutations of inhibitory $GABA_A$ receptor subunit genes *(*GABRA1*,* GABRB3, GABRG2 and GABRD) [5]. Among all different kinds of epilepsy syndromes, our study mainly focuses on Childhood absence epilepsy. Childhood absence epilepsy (CAE) is a hereditary form of epilepsy that usually begins at the age of 4–8 with brief losses of consciousness and frequent staring spells. Genetic faults or mutations associated with CAE have been originated in specialized γ2 subunits of $GABA_A$ receptor channels. Since, it is the prototype of idiopathic, generalized non-convulsive epilepsy [6] with a multi-factorial genetic inheritance. Scientific experiments, described a $GABA_A$ receptor γ2 subunit gene (GABRG2) mutation in a family with classical CAE associated with febrile. The most well characterized γ2 subunit missense mutation is GABRG2 *(R82Q)* associated with childhood absence epilepsy and febrile seizures [7].

In silico molecular-genetic analyses of affected GABA receptors, are used in the identification of potential pathophysiological mechanisms for this type of epilepsy. Here, this study mainly focuses on the normal as well as mutated GABA receptors of CAE. Comparisons of these will help us to reveal the mechanism and pathophysiology of CAE, by using *in silico* techniques.

MATERIALS AND METHODS

The sequences of $GABA_A$ receptor of three subunits (alpha 1, beta 2 and gamma 2) with UniProtKB accession numbers P14867, P47870 and P18507 were retrieved from NCBI (http:/ /www. ncbi.nlm.nih.gov/) in FASTA format.

The gamma 2 subunits of normal and diseased sequences were analyzed for their physicochemical profiles such as molecular weight, Isoelectric point, Net charge, Hydropathy Index, Aliphatic Index and Instability Index by using the tool ProtParam tool (http:/ /web.expasy.org/ protparam/). The main secondary structure elements of the proteins such as α-helices, extended strands, beta turns and random coils were calculated by using protein secondary structure prediction tool GOR IV (http://npsa-pbil.ibcp.fr/ cgi-bin/ npsa_automat.pl?page=npsa_gor4.html). Tertiary structure prediction of normal and mutated (R82Q) gamma 2 subunit was performed using an automated modeling server I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/). The model was validated using the SAVeS server (nihserver.mbi.ucla.edu/SAVES/). The pentamer model was modeled using PatchDock Server (http://bioinfo3d.cs.tau.ac.il/PatchDock/). Molecular visualization tools were used to visualize the structural changes in both, normal and mutated subunits of $GABA_A$ receptor models. The suitable Antiepileptic compounds (72), Anticonvulsant compound (35) and currently approved drugs (17) were selected using NCBI-PubChem (http://pubchem.ncbi.nlm.nih.gov/) chemical databases and molecular properties, drug likeness were calculated by using Molinspiration software server (www.molinspiration.com). The prediction of ADMET is done by using FAF-Drugs2 online tool (http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py?form=FAF-Drugs2#forms::FAF-Drugs2). Schrodinger Glide module was used to analyze the docking mechanisms of the mutated gamma subunit of GABA receptors with the library of ligand molecules.

RESULTS AND DISCUSSION

Primary structure analysis

The gamma 2 subunit of $GABA_A$ receptor protein is stable with an instability index of 39.11 and the protein is hydrophilic inferred from the GRAVY value which is -0.146. The hydrophobicity and polarity at position 371 is minimum and maximum respectively. The average flexibility is maximum and minimum at the positions 382 and 452 respectively. The hydrophobicity is maximum at the

position 343. The polarity is minimum at the position 347. The Childhood absence epilepsy has originated due to a single point mutation (R82Q) in the gamma 2 subunit of $GABA_A$ receptor. The primary structure of the mutated protein was examined but showed no such indifference. The protein is stable with instability index 39.4 and its hydrophilicity deduced from the GRAVY value was -0.144 (Table. 1). The hydrophobicity, polarity and average flexibility are the same as explained above.

Table1: The physicochemical properties of gamma 2 subunit of $GABA_A$ receptor.

Physicochemical properties	Normal	Mutated (R82Q)
Mass	54.12 Da	54.78 Da
Isoelectric point	8.6	8.49
Net charge	15	14
Hydropathy Index	-0.146	-0.134
Aliphatic Index	84.05	84.05
Instability Index	39.11	40.06

Table 2: **The secondary structural composition of gamma 2 subunit of $GABA_A$ receptor**

Secondary structure elements	Normal	Mutated(R82Q)
Alpha helix	28.69%	29.98%
Extended strand	21.63%	23.34%
Beta turn	4.71%	3.43%
Random coil	44.97%	43.25%

Secondary Structure analysis

The amount of the secondary structure content has shown divergence between normal and diseased protein (Table. 2) by GOR method [8]. Predicting the protein secondary structural elements is essential in understanding its structure and ultimately its function. The changes in the secondary structure of a protein, will illustrate their folding, exposed residues, antigenic regions, plan sub-cloning experiments, etc.

Protein model quality

The gamma 2 subunit of $GABA_A$ receptor was modeled using I-TASSER server [9], since the identified templates had very low percentage identity (20%). Since, the identity is less than 30%, hence it is hard to identify the best template and generate accurate sequence-template alignments [10, 11]. The diseased and normal protein models (Table. 3) were analyzed by using the SAVeS (Structural Analysis and Verification Server) (http://nihserver.mbi.ucla.edu). The PROCHECK [12], results of model were nearly satisfied with the standard parameters, where 95% of residues have the backbone geometry falling in favorable regions of the Ramachandran plot [13].

Ligand library

Many of the plant derived compounds of different classes are found to be potential anticonvulsant compounds such as Cannabidivarin [14] from *Cannabis sativa* and Berberine [15] from *Berberis vulgari* [16] etc. The following anti-epileptic drugs namely Levetiracetam [17], Brivaracetam [18] etc, and the current medications such as ethosuximide, valproic acid, lamotrigine [19],

Carbamazepine and vigabatrin [20] were selected for this study. The ligand library of 124 compounds was screened based on their ADMET properties and Lipinski's rule of five. The molecular properties of the best docked ligands with gamma 2 subunit of $GABA_A$ receptors are listed in the Table. 4.

Molecular Docking

Recent breakthroughs in computational and structural bioinformatics offer solutions to unravel the mechanism of action of CAE. Various *in silico* techniques viz computational modeling, virtual screening and docking were used to evaluate the potential of the lead molecules from various sources to treat CAE. Comparative docking analyses were performed for the small molecules such as berberine, brivaracetam, bumetanide, cannabidivarin and safinamide to find the efficiency of the drug for the treatment of Childhood absence epilepsy. From the docking analysis (Table. 5), it is suggested that cannabidivarin (-5.3), an herbal formulation (Figure.1 & 2) may be a novel drug for the treatment of Childhood absence epilepsy.

Table 4:The molecular properties of the ligands (with good docking score).

Ligands	Molecular Weight [g/mol]	XLogP3	H-Bond Donor	H-Bond Acceptor
Cannabidivarin	286.408997	5.97	2	2
Berberine	336.36122	3.6	0	4
Butanamide	364.4161	2.8	3	7
Brivaracetam	212.2887	1	1	2
Levetiracetam	170.20896	-0.3	1	2

Table 5: Docking interactions of top ranking ligands with gamma 2 subunit of $GABA_A$ receptors.

Compound	G score (Kcal/Mol)	Interacting residues
Cannabidivarin	-5.3	Phe77, Met 130, Cys190, Trp82
Berberine	-4.5	Cys283,408, Arg114
Butanamide	-5.3	Asp 161, Cys414,342
Brivaracetam	-5.9	Cys204, Met 130, Cys283
Levetiracetam	-3.2	Lys 41, Trp 82, Cys 283

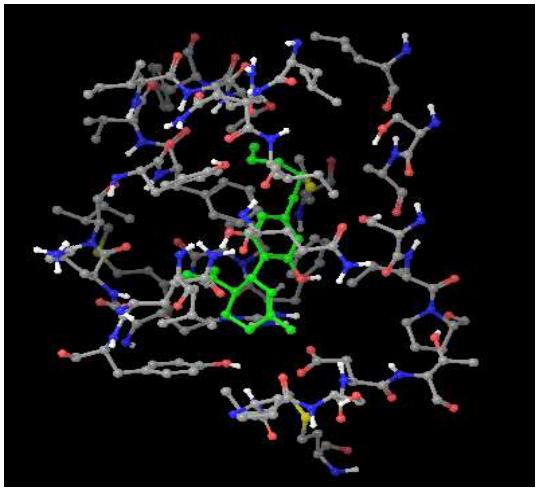

Fig. 1: The Molecular interaction of gamma 2 subunit of $GABA_A$ receptors with the ligand cannabidravin.

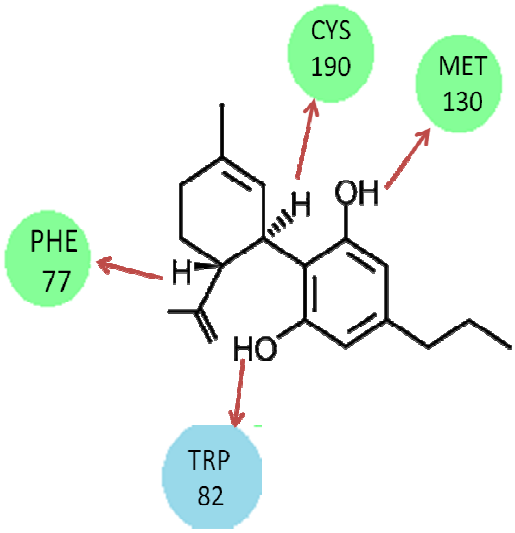

Fig. 2: The amino acid interactions - gamma 2 subunit of $GABA_A$ receptors with the ligand cannabidivarin.

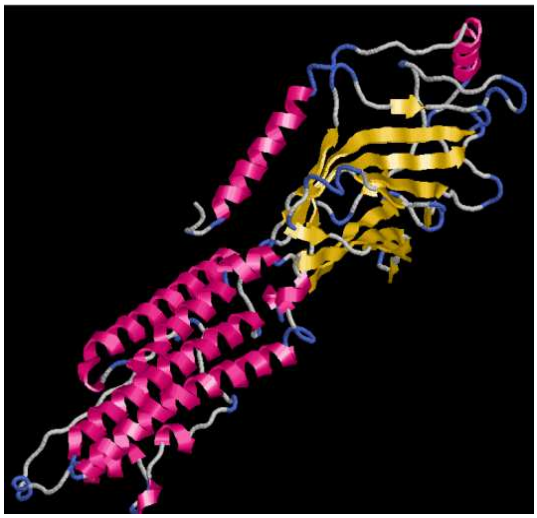

Fig. 3: Modeled structure of (Normal) gamma 2 subunit of $GABA_A$ receptor

Fig. 4: Modeled structure of mutated (R82Q) gamma 2 subunit of $GABA_A$ receptor

CONCLUSION

From this study, it is concluded that the plant compound Cannabidivarin from *Cannabis sativa* could be a promising lead for Childhood absence epilepsy (CAE). Virtual screenings of small molecules (12 compounds) exemplify that the plant derived compounds would inhibit the activity of gamma 2 subunit of $GABA_A$ receptors and control the childhood absence epilepsy syndrome. From these studies we conclude that **cannabidivarin** would be the potent inhibitor against gamma 2 subunit of $GABA_A$ receptors by forming a maximum of number of interactions with the docking score of -5.3. Further, this promising lead molecule has to be validated through various bioassays.

ABBREVIATIONS

CAE, Childhood absence epilepsy; CBDV, Cannabidivarin; JME, Juvenile Myoclonic Epilepsy; FS, Febrile Seizures; GEFS+, Generalized Epilepsy with Febrile Seizures plus; DS, Dravet syndrome; SMEI, Severe Myoclonic Epilepsy in Infancy; GABRA1, Gamma-Aminobutyric acid A receptor Alpha1; GABRB3, Gamma-Aminobutyric acid A receptor Beta3; GABRG2, Gamma-aminobutyric acid A receptor Gamma2; GABRD, Gamma-Aminobutyric acid A receptor delta; GOR, Garnier-Osguthorpe-Robson; GRAVY, Grand average of hydropathy; I- TASSER, The Iterative Threading Assembly Refinement; SAVeS, Structural Analysis and Verification Server; NIH, National Institutes of Health; NCBI, National Center for Biotechnology Information; ADMET, Absorption, Distribution, Metabolism and Excretion – Toxicity; FAF, Free ADME-Tox Filtering .

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