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COMPUTATIONAL ANALYSIS OF INTERACTIONS BETWEEN ANTI-EPILEPTIC DRUGS AND IMPORTANT PLACENTAL PROTEINS-A POSSIBLE ROUTE FOR NEURAL TUBE DEFECTS IN HUMANS

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ABSTRACT

Objective: The reason behind the occurrence of Neural Tube Defects (NTDs) in pregnant women treated with certain Anti-Epileptic Drugs (AEDs) such as Carbamazepine, Valproate, Lamotrigine, Phenobarbital, etc., is not yet known. The relationship between Folic Acid intake and NTDs is not yet established. Folate receptors play a critical role in mediating placental transport of maternal folate to the foetus. Another important protein is Carnitine O-acetyltransferase that is involved in the transport of carnitine, which is much needed for foetal metabolic functions and tissue development. The objective of this study is to understand the interaction of AEDs with two important placental proteins through a docking approach to establishing a suitable explanation of AED's role in NTD.

Methods: A generic algorithm based docking was used to identify and study the mode of interactions between the drugs and placental proteins. For comparison purpose, the natural ligands of these receptors have also been included in the dataset containing AEDs.

Results: Both bonded and non-bonded interactions were observed between AEDs and the crucial residues of these proteins. The drugs formed complex with these proteins with satisfactory binding energy. Some amount of Electrostatic interaction was also observed among a few pairs of protein residue and drug molecules.

Conclusion: We suggest that these drug-protein associations, involving bonded and non-bonded interactions, could be a possible portal by which certain AEDs induce NTDs in the foetus. Higher interaction of Pantothenic Acid with Folate Receptor could be a mechanism through which Pantothenic Acid inhibits Valproic Acid-induced NTDs. And thus, its supplementation can specifically prevent Valproic Acid-induced NTDs. The above mechanism also explains how increased intake of Folic Acid during pregnancy can reduce the occurrence of NTDs.

Keywords: Neural Tube Defect, Anti-epileptic drug, Placenta, Folate transporters, Docking, Carnitine O-acetyltransferase

INTRODUCTION

Neural Tube is a precursor to the Brain and Spinal cord in an Embryo. The Neural tube begins being formed after 21 d (3 w) after fertilization by a process called Neurulation. Neural tube formation is complete by the 4th week after fertilization [1]. NTDs are common congenital malformations that occur when the embryonic neural tube fails to close properly during the first few weeks of development. The reported NTD incidence in India varies from 0.5 to 11/1000 birth [2]. NTDs may be broadly classified into Spina Bifida and Anencephaly. Spina Bifida refers to incomplete development and fusion of one or more vertebral arches with associated involvement of the posterior neural tube. Anencephaly results from failed closure of the anterior neural tube, with the absence of the bones of the cranial vault and absent or rudimentary cerebral and cerebellar hemispheres and brainstem [3].

Epilepsy is a brain disorder involving repeated, spontaneous seizures of any type. Seizures, or convulsions, are episodes of disturbed brain function that cause changes in attention or behaviour. They are caused by abnormally excited electrical signals in the brain. The vast majority of patients suffering from seizures requires daily AED therapy, and in most instances, this is a lifelong treatment. This also holds true for epileptic women falling within the reproductive age group [3]. 50 million people worldwide are affected with epilepsy and half of these are women. There are approximately 2.7 million women with epilepsy in India, and 52% of the women are in the reproductive age group (15-49 y) [4]. For the most part, the clinical course of pregnancies in women with epilepsy is uneventful, with most children born free from either structural or behavioural abnormalities. However, given the *in utero* exposure to AEDs, these children are at a greater risk of being born with birth defects [3]. Commonly used AEDs include Carbamazepine, Phenytoin, Lamotrigine, Levetiracetam, Phenobarbital, Topiramate, Valproate and Vigabatrin [3, 5].

Folic acid is the fully oxidized mono glutamyl form of water-soluble vitamin B9 that is used commercially in supplements and in fortified foods. Metabolically, folic acid is converted to coenzyme forms required in various one-carbon transfer reactions, including synthesis, conversion and modification of nucleotides, amino acids and other essential compounds. Folic acid supplements taken periconceptional have been definitively proven to reduce the risk of neural tube defects significantly. Human Folate Receptors (FRs) are high-affinity receptors that transport folate via endocytosis. They thus play a critical role in mediating placental transport of maternal folate to the foetus [6]. Limiting the availability of folic acid to cells within the embryo will compromise normal growth and development of the embryo [7].

Another important compound is L-carnitine, which is highly polar and plays an important role in transferring the long chain fatty acids across the inner mitochondrial membrane, the detoxification of toxic acyl moieties and the regulation of apoptosis and oxidative stress. Carnitine is important in the foetus for the maturation of carnitine reserve, metabolic functions and tissue development, especially the neural tube [8]. Carnitine O-acetyl transferase (CRAT) helps to transport carnitine from mother to foetus.

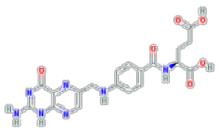
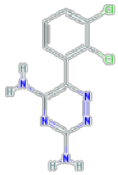
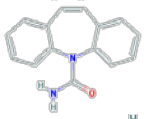
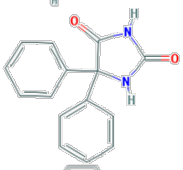
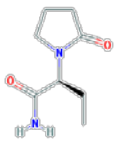
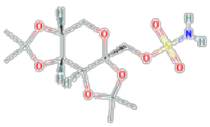
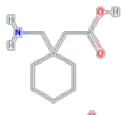
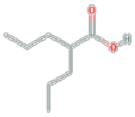
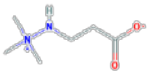
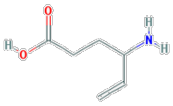
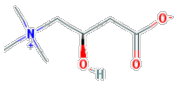
So far, no study has concluded the way by which the AEDs cause NTD in the foetus. Placental proteins that can be specifically associated with NTDs have also not been stated as a fact. Through this study, we have attempted to find a mechanism that would probably be adopted by the AEDs to cause NTD and to hypothesize proteins involved in neural tube defects. Based on the extensive literature work, we have considered two important placental proteins Human Folate Receptors and Carnitine O-Acetyl transferase for our study. A small dataset

comprising of selected AEDs and natural ligands to the proteins were considered. Our paper further aims to explain a route by which Pantothenic Acid inhibits Valproic Acid-induced NTDs. The interaction between the proteins and the ligands has been quantified with a computational approach of docking.

Table 1: Accession numbers of protein data used in this study

Protein name	Uniprot ID	PDB ID
Folate Receptor [11]	P14207	4KN1
Carnitine O-Acetyl Transferase [12]	P43155	1S5O

Table 2: Information about ligand structures obtained from PubChem

Compound name	2D Structure	PubChem ID	Molecular weight (g/mol)
Folic Acid		CID_6037	441.397460
Pantothenic Acid		CID_6613	219.234980
Lamotrigine		CID_3878	256.091380
Carbamazepine		CID_2554	232.26858
Phenytoin		CID_1775	252.267980
Phenobarbital		CID_4763	232.235280
Levetiracetam		CID_5284583	170.20896
Topiramate		CID_5284627	339.362040
Gabapentin		CID_3446	171.236780
Valproate		CID_3121	144.211440
Mildronate		CID_123868	146.187560
Vigabatrin		CID_5665	129.157040
Carnitine		CID_85	162.206840

MATERIALS AND METHODS

Works of literature were read to identify proteins that could be required for neural tube formation and closure. The dataset comprised Lamotrigine (LMT), Pantothenic Acid (PA), Carbamazepine (CBZ), Phenytoin (PHT), Phenobarbital (PB), Levetiracetam (LEV), Topiramate (TPM), Gabapentin (GBP), Valproate (Valproic Acid-VPA), Mildronate (MI) and Vigabatrin (VGB) along with natural substrates [Folic Acid (FA) and Carnitine (CA)] [3]. Protein information was obtained from Uniprot Database [9]. Protein structures were retrieved from the Protein Data Bank (PDB) [10] and Ligand structures from PubChem.

The interactions between proteins and ligands were studied using iGEMDOCK, a Graphical Environment for Recognizing Pharmacological Interactions and Virtual Screening. It is easy-to-use software for docking, virtual screening, and post-screening analysis. The pharmacological interactions represent conserved interacting residues that often form binding pockets with specific physicochemical properties to play the essential functions of the target protein [13]. Molecular graphics and analyses were performed with the UCSF Chimera package. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco [14].

RESULTS AND DISCUSSION

As per the crystal structure, folic acid binds to amino acids such as R119, D97, Y101, W118, W187, W156, W154, H151, G153 and S190. It was observed that the folic acid prefers a binding pocket rich in hydrophobic amino acid, tryptophan, with which it exhibited both bonded and non-bonded interactions. The docking results reflected the favourable interactions between drugs and crucial residues of FR which are evident from the docking energies as tabulated below in table 3 and as shown in fig. 1. The interaction energy of the ligand with the protein is the sum of the Vander Waals Force, electrostatic energy and energy through Hydrogen bonds. In tables, Residues showed as italics exhibit electrostatic interaction with the amino acids while the residues represented as bold exhibit Vander Waals interaction. The other residues exhibit Hydrogen bonded interaction. More negative the total energy, better the interaction between the protein and Ligand/Drug. In this case, the van der Waals interaction contributed more to the binding energy than the other interactions. Most of the ligands interacted with the tryptophan residues present in the active site. Drugs like VPA, GBP, MI and VGB docked with the basic amino acid R119 through electrostatic attraction and hydrogen bond with its side chain. FA exhibited only Vander Waals interaction with the side chain of R119 and it did not show electrostatic interaction with any of the active site residues.

Table 3: Energy of the interaction of drugs with Folate receptor and critical residues involved in the interactions

Drug Name	Total Energy	Vander Waal	HBond	Elec	Interacting Residues
Folic Acid	-175.5	-136.51	-38.67	-0.33	D97 W118 W154 W156 W187 S190 **D97 Y101 W118 H151 W156 W187**
Lamotrigine	-108.9	-81.43	-27.47	0	D97 S190 **D97 Y101 W118 H151 W156 W187**
Carbamazepine	-107.2	-100.31	-6.928	0	**D97 Y101 W118 H151 W187**
Phenytoin	-101.1	-80.56	-20.54	0	D97 T98 **D97 Y101 W118 H151 W156 W187**
Phenobarbital	-98.3	-80.48	-17.81	0	D97 T98 **D97 Y101 W118 W187**
Pantothenic acid	-97.3	-64.02	-27.49	-5.74	*R119* D97 W187 **D97 Y101 W118 H151 W187**
Levetiracetum	-92.1	-67.45	-24.64	0	D97 Y101 W187 **D97 Y101 W187**
Topiramate	-86.1	-68.03	-18.05	0	D97 W187 S190 **D97 Y101 Q118 H151 W187**
Gabapentin	-80.3	-56.47	-18.74	-5.06	*R119* **D97 Y101 W118 H151 W187**
Valproate	-77.2	-57.60	-13.91	-5.67	*R119* **D97 Y101 W118 H151 W187**
Mildronate	-73.4	-56.90	-10.35	-6.18	*R119* **D97 Y101 W118 H151 W187**
Vigabatrin	-72.8	-49.61	-17.84	-5.32	*R119* D97 **D97 Y101 W118 W187**

Table 4: Energy of the interaction of drugs with carnitine O-acetyltransferase and critical residues involved in the interactions

Drug Name	Total Energy	Vander Waal	HBond	Elec	Interacting Residues
Topiramate	**-98.1**	-68.04	-30.05	0	H322 T444 **H322 Y431 S433**
Phenytoin	**-94.7**	-75.17	-19.52	0	S433 **H322 Y431 S433**
Lamotrigine	**-88.5**	-60.35	-28.17	0	**H322 Y431 S433**
Carbamazepine	**-87.6**	-75.64	-11.93	0	H322 S433 **H322 Y431 S433**
Pantothenic acid	**-85.5**	-55.01	-28.88	-1.58	*H322* H322 **H322 Y431 S433**
Phenobarbital	**-80.2**	-60.63	-19.56	0	H322 S433 **H322 Y431 S433**
Gabapentin	**-68.2**	-48.32	-18.85	-1.01	*H322* H322 **H322 Y431 S433**
Carnitine	**-67.3**	-46.28	-19.55	-1.51	*H322* H322 **H322 S433**
Levetiracetam	**-66.4**	-53.78	-12.64	0	H322 **H322 Y431 S433**
Vigabatrin	**-63.2**	-44.20	-18.18	-0.82	*H322* H322 **H322 S433**
Mildronate	**-62.8**	-46.76	-14.36	-1.62	*H322* H322 **H322 S433**
Valproate	**-61.4**	-45.11	-15.00	-1.31	*H322* H322 **H322 Y431 S433**

It was noted that the vitamin PA, which has been proved to decrease the VPA-induced neural tube defects, showed interactions similar to VPA, but the binding energy was higher for PA [5]. This could be a factor of FR's preference to PA over VPA. Another noted interaction of PA was hydrogen bond and van der Waals interactions with the side chain of acidic amino acid D97 which were not exhibited by VPA. Docking results showed that PA bound with crucial residues more strongly than the AEDs which are evident from the overall energy as well as the contribution from each interaction. This could possibly be a valid reason for the PA to be preferred by the receptor than the other. The interactions shown by AEDs to the active site residues show that the AEDs especially VPA could possibly act as antagonists to FR.

The crystal structure of CRAT reveals that carnitine binds to a pocket that includes amino acids H322, T454, Y431, S433, E326, W81, V548, T532 and F545. Table4 describes the results of interactions between the drugs and CRAT. Few important drug-receptor interactions are revealed in fig. 2. Docking results showed that TP, PH, LMT, CBZ, PHB and GBP bound with carnitine receptor stronger than carnitine with lower binding energies.

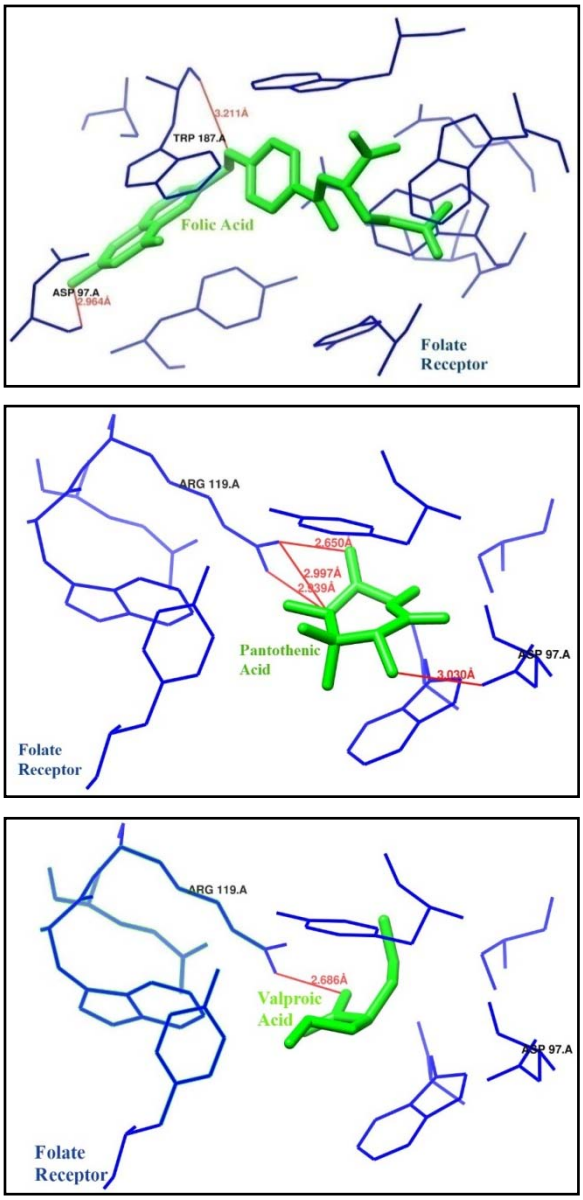


Fig. 1: Bonded interactions of Folic acid, Pantothenic acid and Valproate with Folate Receptor. H-bonds are shown as red lines

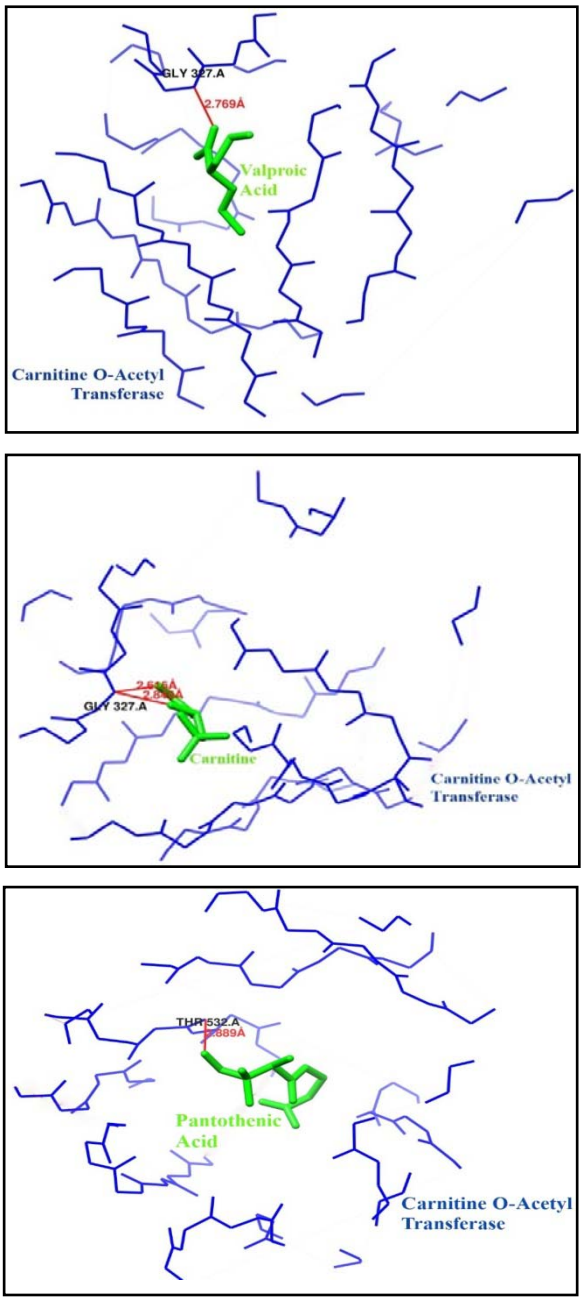


Fig. 2: Bonded interactions of Carnitine, Pantothenic acid and Valproate with Carnitine O-Acetyltransferase. H-bonds are shown as red lines

Most of total energy derives from the van der Waals interactions of drugs with the protein. Docking results showed that VPA, GBP, MI and VGP showed an electrostatic attraction to H322, which is one of the crucial residues. Natural ligand, carnitine also showed an electrostatic attraction to H322. The highest Electrostatic Static energy with the overall protein was shown by the PA and Carnitine. PA binds to many amino acids such as Y431, S433, S531, T532 and S533 to which VPA fails to bind. PA's interaction with CRAT is stronger than VPA and carnitine as evident from the interactions and binding energy. These reasons could explain why PA is preferred over VPA, by CRAT.

As shown by the dock scores, the hypothesis implying a preference of the protein to bind via bonded and non-bonded interactions give a possible explanation to the antagonistic/competitive binding of drugs to the protein. No substantial proof is available to associate specific proteins to Neural Tube Defects. Based on the results, we hypothesize FR and CRAT as potential targets that could play a

prominent role in the Neural tube formation and their inhibition by AEDs is associated with the occurrence of NTDs.

CONCLUSION

Our study shows that Neural Tube Defect causing Anti-Epileptic drugs shared potential interactions with two important placental proteins, Folate receptor and Carnitine O-Acetyltransferase. The binding of the Anti-Epileptic drugs with these crucial proteins makes them inefficient in their routine function thus resulting in Neural Tube Defect. This work suggests that these two placental proteins could play a significant role in Neural Tube formation. Both Valproate and Pantothenic acid compete for the active site of these two proteins as validated by docking. Based on the computational method, it is concluded that the Pantothenic acid binds to these receptors strongly than the Anti-Epileptic drugs suggesting the preference of it over other drugs. This competitive mechanism adopted by Pantothenic acid in binding to these proteins could be a strong rationale of reduction in valproate-induced Neural Tube Defect risks in patients under compulsory valproate treatment. This rationale derived from the computational method is much in understanding with the experiments done with Folic acid and carnitine [15, 16]. Thus, studying the effects of various drugs on these placental proteins can help to prevent their teratogenicity by reducing their risks of inducing Neural Tube defects. This work is the first of its kind to explain this fact through computational method.

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CONFLICTS OF INTERESTS

All authors have none to declare.

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