Structural insights into the caffeine-based inhibition of Palmitoleoyl-protein carboxylesterase

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INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disorder which is the most common form of dementia that starts slowly and gradually worsens over time[1]. The exact reason of the disease is not clearly understood but is observed that the brain proteins (amyloid, tau) will fail to function normally and interfere with the functions of neurons by getting deposited and forming plaques[2] (figure 1). Thus, interfering with cognitive and non-cognitive behavioural functions[3]. While there is no cure or a way to prevent or slow down the progression of the disease there are few drugs and therapies to minimise its symptoms. Cholinesterase inhibitors like Razadyne, Exelon, Aricept are used to treat mild to moderate Alzheimer's. Drugs like Namenda, Aricept, Exelon and Namzaric are used to treat moderate to severe alzheimer's[4]. Although there's been a progress in understanding the basic biology of the disease, the early detection of it is yet to be discovered. This is believed to be one of the reasons due to which the the drugs that are being given to the patient in advanced stages are not being able to show their effectiveness in treating the disease. There are some ongoing clinical trials such as stem cell transplantation methods, regenerative medicine in AD mouse models to come up with new therapies for AD[5].

Wnt signalling pathways are a group of pathways that mediate cell to cell signaling and help in adult tissue homeostasis. It is useful for neuronal differentiation, development and neural stem- cell maintenance in the nervous system. Notum, palmitoleoyl protein carboxylesterase is a protein that in humans encoded by notum gene which acts as key negative regulator of Wnt signalling pathway by causing the delipidation of Wnt ligands thus causing a series of misrecognition events,resulting in inefficiency of the ligands to bind to receptors. It is observed that caffeine inhibits notum activity thus acting as a potent notum inhibitor by binding at the catalytic pocket of the protein[6][7] (figure 3). The severity of AD is high in people aged 65 and more. It is estimated that 5.8 million Americans under this age group have AD today. 122,019 deaths from AD are officially recorded in 2018 making it the sixth leading cause of death in united states[8]. Thus, more advancements need to be made in order to control the mortality of the disease.

EXPERIMENTALS

This notum protein is downloaded from PDB data bank (rcsb.org) with a PDB ID - 6TV4 under the title CFF- Notum complex. It is classified as a signaling protein and homo sapiens serve as its expression system. To narrow down the crystal structures filters have been used.

filters used- Refinement filter : Homo sapiens
Experimental method : X-ray diffraction
Refinement resolution: 1.5-2.0

It has an R- value free of 0.214 A^0 and R- value work of 0.170 A^0 with a resolution of 1.53 A^0. later on, the quality of the structure was determined using two tests. The secondary structure analysis of the protein was performed using PYMOL. It has a macromolecule, Palmitoleoyl-protein carboxylesterase NOTUM with an entity ID:1.

It also has five small molecules (ligands) namely NAG, CFF, SO4, GOL and DMS. Some of the ligands form hydrogen bonds with the protein which is analysed using PYMOL.

Laguipo, 2019

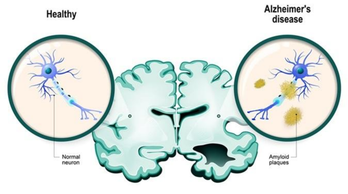

Figure1: Representation of healthy brain and Alzheimer's infected brain showing healthy neurons and neurons with amyloid plaques respectively

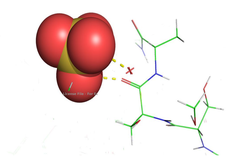

Figure 2: Sulphate molecule forming two hydrogen bonds with serine of NOTUM protein and water molecule respectively

Buechler et al, 2018

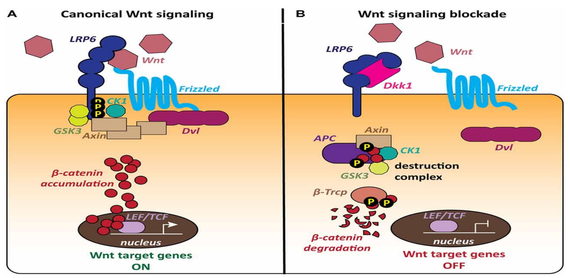

Figure 3: Comparison between normal and deficient Wnt signaling pathway

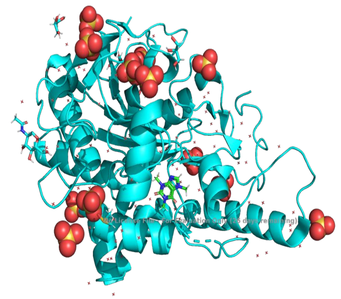

Figure 4: Caffeine- based inhibition of Palmitoleoyl- protein carboxylesterase

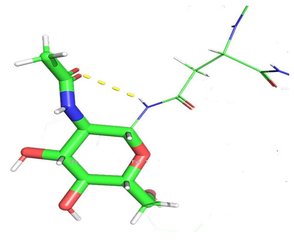

Figure 5: NAG ligand forming a hydrogen bond with the asparagine of palmitoleoyl- protein carboxylesterase

RESULTS & DISCUSSION

In the first test that is conducted to determine the quality of protein where the difference between R- value free and R- value work should be less than or equal to 0.05 (5%) and we got a value of 0.044 (<0.05) hence it passed the first test. In the second test , R- value work should roughly be equal to 1/10th of its resolution i.e., 0.153 A^0 but we got a value of 0.17 A^0 which is roughly equal to 0.153 A^0. Thus, it passed the second test. By performing the secondary structure analysis we came to know that

Number if alpha helices: 11
Number of beta strands: 14

The small molecule CFF has no hydrogen bonds hence it is a hydrophobic molecule. NAG' 605 forms one hydrogen bond with nitrogen at delta 2 position of asparagine of NOTUM protein (figure 5) and SO4' 615 formed two hydrogen bonds one with water molecule and other with serine of NOTUM protein (figure 2).

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