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Gamma-Linolenic Acid Interactions With The Human Voltage-Gated Sodium Channel 1.7 By Molecular Docking: Its Role In The Action Mechanism On Mastalgia

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Abstract:
Background*: Mastalgia is the main complaint in clinical consultant and its management is otherwise. The evening primrose oil (EPO) has been used to treat mastalgia. EPO contains linoleic acid and gamma-linolenic acid (GLA) that might modulate the immune system and improve breast pain. Voltage-gated sodium channels (Na_V) have been postulated as analgesic targets in humans and the sodium channels $Na_V1.7$ mainly the most important role. Thus, to investigate whether GLA might be able to interact with the $Na_V1.7$.*
Materials and Methods*: A study in silico approach by molecular docking into 3D structure of analogous $Na_V1.7$ and GLA or carbamazepine (CBZ) molecules. The simulations were processed in the Dockthor and analyzed by UCSF Chimera. Binding energy scores of molecules with channel were obtained and compared by Student's t-test, the level $P < 0.05$ was considered significant.*
Results*: The energy scores into GLA or CBZ and $Na_V1.7$ presented good binding activity. However, the energy of CBZ was lower than GLA ($P = 0.012$). The spatial positions showed both were situated on the outer pore domain channel. Regarding binding sites, both molecules were bonded hydrogen with GLU194 and ARG190 residues.*
Conclusion: *The gamma-linolenic acid made interactions with the voltage-gated sodium channel 1.7, similarly to carbamazepine.*
Key Word*: Gamma-Linolenic Acid; Voltage-Gated Sodium Channel; Molecular Docking Simulation*

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I. Introduction

Mastalgia or breast pain is a common cause of anxiety amongst women and frequently associated with primary care clinic consultation[1]. Approximately 40 to 90% of women experience some type of breast pain, and in 23% of the cases, it is severe[2]. The first-line therapy for mastalgia is usually conservative, which involves physical support, over the counter analgesics, and manipulation of hormone-based medication for at least six months. If not responded to, treatment will be upgraded to the second-line therapy, such as tamoxifen and danazol, which may be more effective but not without adverse effects[3].

The seeds of the evening primrose are rich in omega-6 essential fatty (EFAs), including linoleic acid and gamma-linolenic acid (GLA)[4]. Women with breast pain have low levels of GLA and its metabolite. Thus, treatment with evening primrose oil will raise the levels of GLA and its metabolites towards normal and probably relieves breast pain[5]. The therapeutic effects of EPO are attributed to the direct action of its component EFAs on immune cells as well as their indirect effect on the synthesis of eicosanoids (e.g., prostaglandins, cytokines, cytokine mediators), which are significantly high in mastalgia[4]. A multicenter study randomized concluded that GLA efficacy was similar to placebo, regardless of whether antioxidant vitamins were added or not[6]. Other study showed EPO is a safe medication with similar efficacy for pain control in women with mastalgia compared to a placebo, topical NSAIDS, danazol, or vitamin E[7].

A route to treating many different types of pain has been blocking peripheral nerves that lead to suppressing the electrical signals carried by voltage-gated sodium channels (Na_V)[8-10]. Specifically, human-validated analgesic targets such as the sodium channels $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ are of great interest for the

development of new pain therapie[11]. Thus, we decided to investigate whether GLA might be able to interact with the $Na_V1.7$ proposing a new action mechanism.

II. Material And Methods

This experimental study was carried out *in silico* approach. Molecular docking insights understand the function-structure relation in a pharmacological target and its ligand-protein binding[12].

Procedure methodology

The chemical structures of GLA (CID: 5280933) and CBZ (CID: 2554), a drug Na_V blocker, were downloaded from the PubChem database and the 3D structure of analogous $Na_V1.7$ from the PDB database (ID: 6N4I). The channel protein and chemicals were molecularly docked by Dockthor® and classified in order of highest affinity with the channel[13]. The simulations were processed from the grid center coordinates (x = 97,3135, y = 249,151 and z = 214,9925) with 884736 total grid points. The docking poses selected and chemical interactions were visualized by UCSF Chimera version 1.14 (University of California, San Francisco, CA).

Statistical analysis

Data was analyzed using GraphPad Prism version 4.01 (GraphPad Software Inc., San Diego, CA). Student's *t*-test was used to ascertain the significance of differences between mean values of affinity. The level $P < 0.05$ was considered significant.

III. Result

The simulations of GLA and CBZ in the $Na_V1.7$ were 1,000,000 conformers. The best three were selected, because it presented low binding energies that were calculated and shown in Table 1. Our data showed that the binding energies of GLA or CBZ were - 7.34 ± 0.15 and - 8.47 ± 0.27 Kcal/mol, respectively (Table no 1). Those values were compared and the energy of CBZ was lower than GLA, suggesting CBZ was more stable in the $Na_V1.7$.

Table 1: Binding energy scores into GLA or CBZ with the $Na_V1.7$.

Ligand	Affinity (Kcal/mol)
GLA	- 7.34 ± 0.15
CBZ	- 8.47 ± 0.27*

Being: * $P = 0.012$

The spatial positions into GLA and CBZ with the $Na_V1.7$ were presented in Figure 1, which may show a blockade channel mainly GLA that both were situated on the outer pore domain channel (Figure 1C and D). Visualizing the 3D structures both chemicals connected the closed helices of the $Na_V1.7$, however GLA and CBZ presented different affinity.

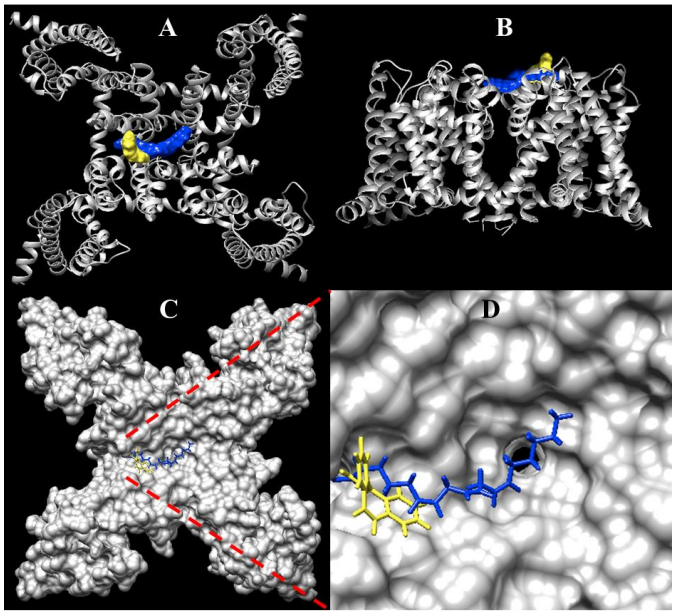

Figure 1: GLA (blue) and CBZ (yellow) binding poses on the Nav1.7. The top (A, C and D) and side (B) views of the channel.

The GLA- and CBZ-binding sites are located and clearly visible (Figure 2) of the $Na_V1.7$, sited in the outer pore domain channel. Hydrophilic interactions were identified and classified, where GLA was bonded hydrogen by GLU194 and ARG190. The distances between the side chain of GLU194/ARG190 and GLA were 1.6 and 1.8 Å, respectively (Figure 2A). Interestingly, CBZ was bonded by the same residues, whose distances were 1.9 and 1.9 Å, respectively (Figure 2B). It suggests both chemicals were pocket similar.

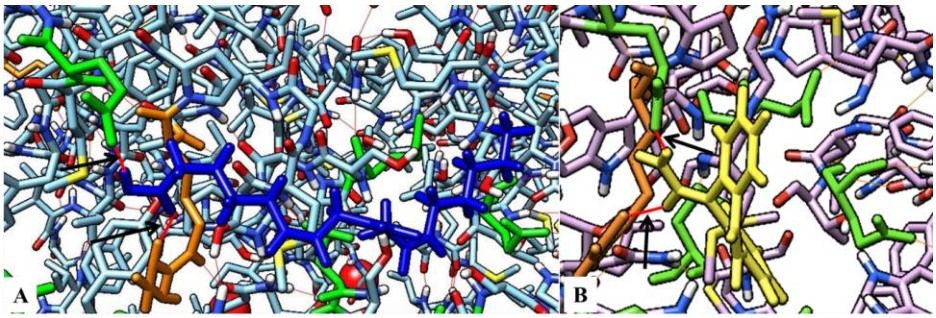

Figure 2: Chemical interactions of the GLA (blue) and CBZ (yellow) with residues in the $Na_V1.7$. The black arrows showed hydrogen-bond (red) with ARG190 (orange) and GLU194 (green).

IV. Discussion

The gamma-linolenic acid is present in large quantities in evening primrose oil and has been found in management of mastalgia.[7] Nevertheless, the mechanism involved in these effects on mastalgia has not been identified. Here, our showed the GLA was able to link in the $Na_V1.7$ and to block to the outer pore domain channel, suggesting that GLA could inhibit this channel.

The GLA is an essential polyunsaturated fatty acid[3] and presents structure available to interact with biological molecules such as proteins. Na_V channels are hetero-multimeric proteins constituted of large ion conducting α-subunits and smaller auxiliary β-subunits[14]. The α-subunit is made up of a single transcript that encodes four 6-transmembrane segment domains, each one of these can be divided into two functional sub-domains known as the voltage-sensing domain and the pore domain[15-19]. Due to the $Na_V1.7$ being a target for the development of new pain therapies[8], we observed molecular docking GLA presented low binding energy with the $Na_V1.7$ as soon as the molecular docking is more stable that presents lower binding energy[20]. This energy score into GLA and $Na_V1.7$ was less than minus 7 Kcal/mol, indicating good binding activity[21].

The most selective and well-known Na_V blocker is tetrodotoxin, a natural product which comes from symbiotic bacteria in the pufferfish (and some other animals') diet[22,23]. Many Na_V modulating compounds are used to treat clinical conditions caused by changes to excitability, such as anticonvulsants (CBZ, phenytoin), local anesthetics (lidocaine), and antiarrhythmics (mexiletine)[24]. Our data showed the GLA was positioned on an outer pore domain in the $Na_V1.7$ that could correspond to the blockade channel similarly to CBZ. In addition, cannabidiol has been found to interact with $Na_V1.7$ in a similar manner[25].

The Na_V pore structure involves a large external vestibule, a narrow selectivity filter, a large central cavity that is lined by S6 segments that is filled with water, and an intracellular activation gate that is formed by the crossing of S6 segments at the intracellular side of the membrane[26-29]. The selectivity filter has a high field strength on the extracellular side which is constituted of amino acid side chains. This outer vestibule is followed by two ion coordination sites that are formed by backbone carbonyls[28,29]. From a functional perspective, both the outer and inner segments of the pore domain are interaction sites for pharmacological agents[30]. We observed a GLA binding site residing on the outer selectivity filter, which is a more rigid part of the Na_V pore domain and in a similar manner to tetrodotoxin[22]. In contrast, another Na_V blocker such as cannabidiol has been sited in the hydrophobic pockets present between subunits that run perpendicular to the channel direction, just below the sodium ion selectivity filter of the voltage-gated sodium channel from *M. marinus*[31-33].

The first evidence that $Na_V1.7$ had a role in peripheral pain pathways came from a conditional knockout study in a subset of mouse sensory neurons expressing another sodium channel, $Na_V1.8$.[34] These sensory neurons have been found to be important for inflammatory pain, and the conditional deletion of $Na_V1.7$ in these cells induced a dramatic loss in inflammatory pain.[34,35] In fact, the $Na_V1.7$ target could be fundamental to treat pain and mastalgia, since pain to be the most common concern of patients presenting with mastalgia. Here, we showed GLA interacted with $Na_V1.7$ and probably a channel blocker that supports its action mechanism for mastalgia treatment in women.

V. Conclusion

The gamma-linolenic acid made interactions with the voltage-gated sodium channel 1.7, similarly to carbamazepine. Those findings reveal its action mechanism may be due to channel blocker that corroborate management of mastalgia. In future, *in vitro* studies will need to reinforce this hypothesis.

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