Assessing the Viability of Peginterferon Alfa-2b Inhibiting Neurotoxin-Ganglioside Binding in Guillain-Barré Syndrome

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Abstract

Guillain-Barré Syndrome (GBS) is a debilitating neurological condition causing damage to the neural plasticity across the peripheral nervous system from an autoimmune response triggered by the binding of pathogen-derived neurotoxins to glycosphingolipid carriers being gangliosides. Though current treatment solely targets such a response post-onset through intravenous immunoglobulin infusion, an approach to suppressing the initial exhibition of GBS derives from ceasing neurotoxins from attachment to gangliosides through administering Peginterferon Alfa-2b, an immunomodulatory drug, as a ligand to attach and block to binding domain proteins to restrict neurotoxin-ganglioside contiguity. Through *in-silico* docking, a protein-ligand complex was manufactured amongst Peginterferon Alfa-2b and *Clostridium botulinum* neurotoxin protein 8AGK, a binding domain known for attachment to GD1a gangliosides. This analyzed the viability in administration and affinity of the Peginterferon ligand in blocking 8AGK's ganglioside-binding capabilities as a feasible primary therapy against this critical syndrome. Assessing binding strength was evaluated by measuring statistical variations in mean distances for components vital to the binding affinity for the Peginterferon including Angstroms between optimal and computed hydrophobic overlapping and hydrogen bonding interactions amongst protein-ligand residues. Furthermore, the bioavailability, lipophilicity, solubility, and chemistry of the Peginterferon using SwissADME were assessed for feasibility in real-world therapeutic applications. Through evaluating such components, results demonstrated that Peginterferon Alfa-2b was effective as a pharmaceutical to combat GBS for its strong binding affinity of maximizing Van der Waals contacts whilst minimizing steric hindrance coupled with desirable pharmacokinetics for real-world application. Such findings underscore the potential for a novel, proactive approach to treating GBS.

Introduction

Overview

Guillain-Barré Syndrome (GBS) affects over 100,000 people worldwide yearly, with the only current course of treatment offered post-onset after the neuropathic repercussions begin to exhibit, causing the complete demyelination of nerve fibers across the peripheral nervous system [1]. The immune-mediated response is responsible for triggering these debilitating processes due to the production of antibodies against gangliosides (AGAs), an autoimmune response targeted towards one's gangliosides. Gangliosides are key molecules composed of glycosphingolipids, serving to protect the nervous system from immune attacks. However, in common GBS-inducing pathogens such as *Campylobacter jejuni*, gangliosides invert in function, acting instead as receptors for neurotoxins to become autoimmune targets [2]. This process of inversion in ganglioside function is depicted in the following figure demonstrating the mechanism

of the *Clostridium botulinum*, a GBS-mimicking pathogen as it instigates the syndrome into effect through this viral infection (Figure 1).

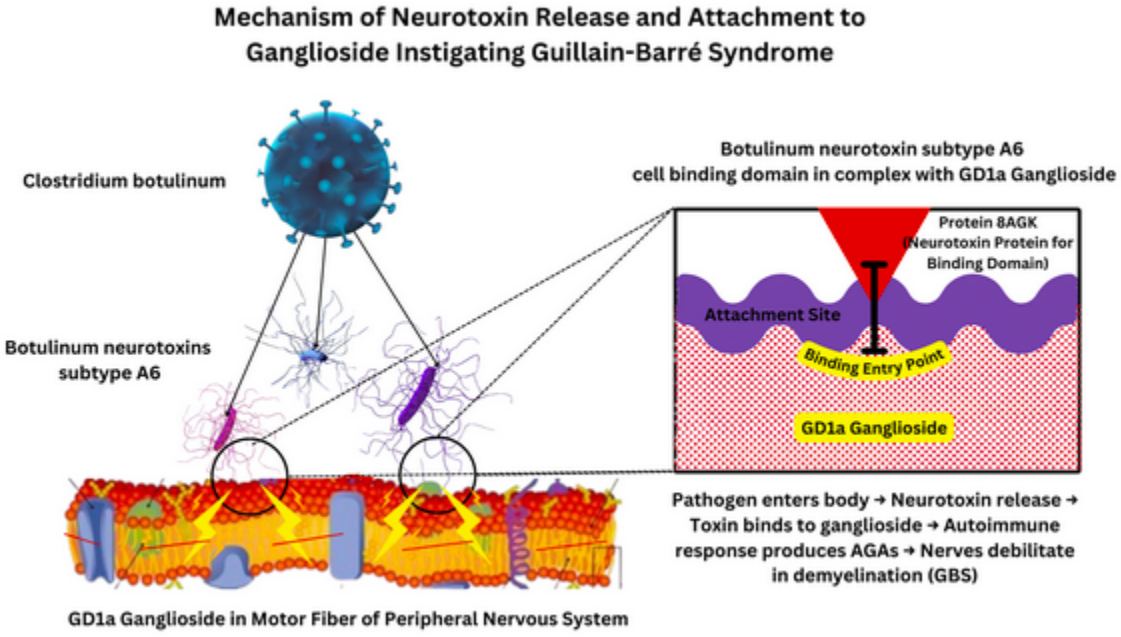

Figure 1. Mechanism of Neurotoxin Release and Attachment to Ganglioside Exhibiting Botulism-Mimicking GBS

An apparent toxin affiliated with binding to GD1a gangliosides, being the primary ganglioside for maintaining neural plasticity and aiding in motor nerve function, is Botulinum neurotoxin type A6. This neurotoxin derives from the aforementioned *Clostridium botulinum*, a pathogen causing botulism that mimics the mechanism of GBS [3]. Botulism is a condition that classifies as a subcategory under GBS-derived conditions, thus denoting the mode of infection caused by *Clostridium botulinum* to mirror that of the typical GBS-inducing pathogen [4].

Scientific Question

As of now, the only form of treatment applied is through intravenous immunoglobulin infusion (IVIG) and is only used post-onset when GBS has fully taken effect. However, a possible solution to treat GBS before when the condition has taken complete effect is by intervening in the source of the infection itself from allowing AGAs to be further created. By mobilizing a drug ligand to attach to the binding domain protein 8AGK of the *Clostridium botulinum* neurotoxin to prevent it from further attaching to such gangliosides, the autoimmune response may not stimulate any further. In turn, this will cause AGAs to diminish in production and inhibit the effects spurred by GBS. Such a drug ligand being potentially feasible for application in such a circumstance is Peginterferon Alfa-2b due to it being a PEGylated compound, which contributes to strengthening immunogenicity to contest GBS' autoimmune attack response and has a prolonged presence in the bloodstream due to the addition of a polyethylene glycol (PEG) chain to the interferon (Figure 2). The presence of PEGs outweighs other residues' compatibilities with other ligands due to the abundance of available binding residues of PEGs in protein 8AGK in contrast to glucose (GLC), gamma-linolenic acid (GLA), sialic acid (SIA), and ethylene glycol (EDO) (Figure 3). Thus, the question being determined in the course of this research is: Is

Peginterferon Alfa-2b a viable drug for a pharmaceutical treatment to suppress the effects of GBS?

Figure 2. Presence of Polyethylene Glycol (PEG) in the Center Chain of Neurotoxin Protein 8AGK Pre-Docking for Compatibility with Drug Ligand Peginterferon Alfa-2b

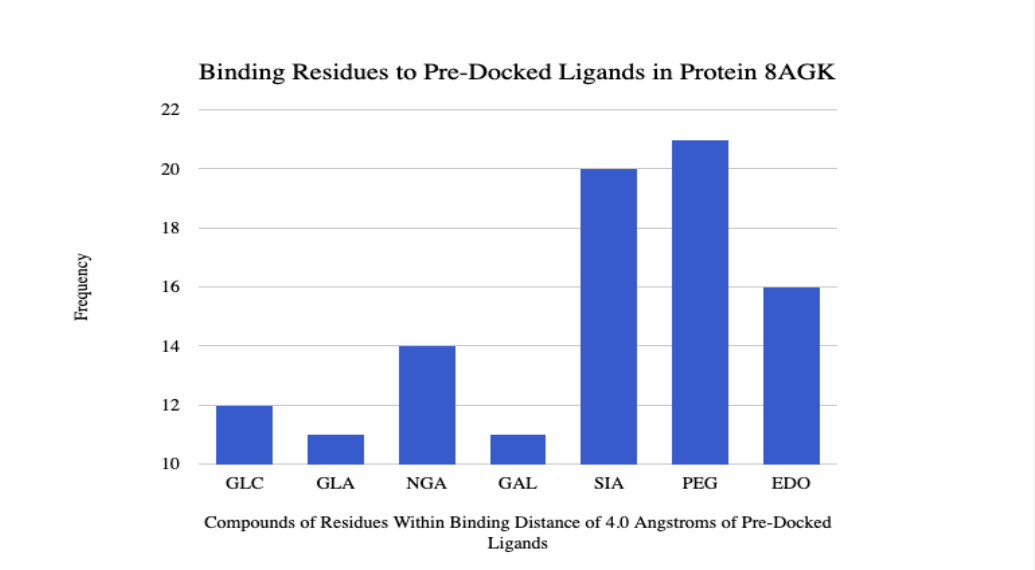

Figure 3. Binding Residues to Pre-Docked Ligands in Protein 8AGK for Docking Compatibility

Goals

To evaluate the feasibility of Peginterferon Alfa-2b, a computational docking was performed among the protein and ligand to create a protein-ligand complex which can be analyzed to assess the potential in drug delivery and binding affinity of the ligand as a course of early treatment against GBS. This computational complex would replicate the optimal bonding of the Peginterferon to the binding protein of the neurotoxin, allowing us to assess the capability in the Peginterferon to inhibit the neurotoxin from attaching to GD1a gangliosides in the peripheral nervous system. With a strong binding affinity through hydrogen bonding interactions along with a firm stability created through hydrophobic overlapping among the protein and drug ligand, the Peginterferon can effectively inhibit the expression of GBS from further arising as it restricts the neurotoxin's binding sites from being able to attach to gangliosides. The other component to be achieved in determining the viability of Peginterferon Alfa-2b is the administration of the drug itself, as determined through quantitative analysis of the drug's oral bioavailability, pharmaceutical solubility, and lipophilicity. Achieving the necessary goals of high water solubility, favorable pharmacokinetics, and maintaining hydrophilic tendencies, along with achieving a strong molecular binding affinity will have demonstrated Peginterferon Alfa-2b to be an applicable therapy to combat GBS.

Methodology

The computational docking process and quantitative analysis of the drug's viability were entirely performed on a computer with no other materials. To prepare neurotoxin protein 8AGK for docking through DockingServer, buffer molecules were removed and hydrogen molecules were added to achieve replicating the most realistically simulated binding affinity and orientation of the ligand. Data was collected for detecting hydrophobic clashes among already-bound ligands of their overlapping distances among such regions and the distances between such residual regions in Angstroms. Such data was assessed for the stability and non-competitive binding properties of the protein-ligand complex to minimize hindrance from steric clashes and was determined through one-sample μ tests for a difference among the mean overlapping distance and interatomic distance to the respective optimal distances of 0.4 Å and 2.5 Å for maximization of Van der Waals contacts without further exhibiting steric hindrance. Similarly, through UCSF ChimeraX-1.8, the Alfa-2b ligand was processed before docking by adding the necessary Gasteiger charges and hydrogens. Using SwissDock, the protein and ligand underwent docking to create a bonded protein-ligand complex that retrieved 20 models of varying binding affinities. A flowchart is depicted in the following figure to illustrate the workflow in the series of computational software used to construct the protein-ligand complex amongst binding protein 8AGK and the Peginterferon Alfa-2b ligand (Figure 4).

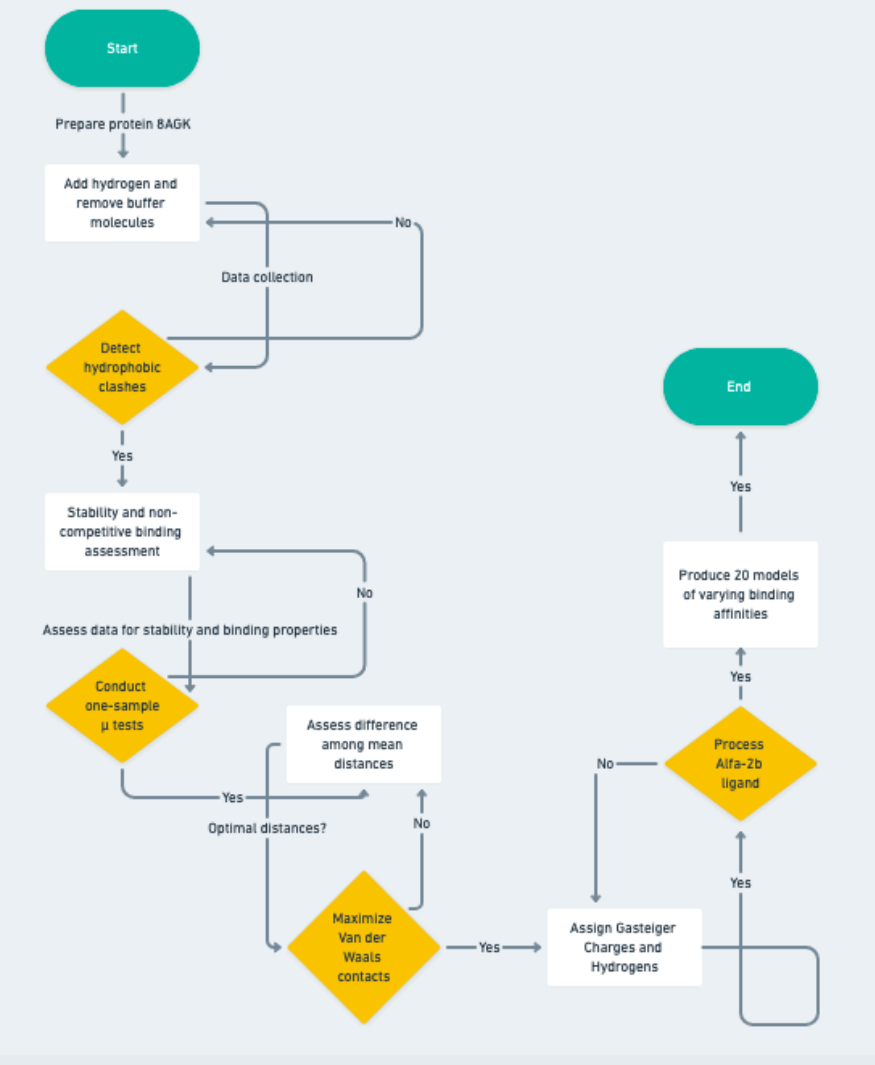

Figure 4. Workflow of Computational Docking between Binding Domain Protein 8AGK and Peginterferon Alfa-2b Ligand

For assessing the viability in drug delivery of Peginterferon Alfa-2b, a precise SwissADME analysis was computed, producing data for the drug's lipophilicity, water solubility, pharmacokinetics, druglikeness, and medicinal chemistry. A linear regression was determined in finding the experimental solubility of Peginterferon Alfa-2b predicted from the initial solubility computation accessed through the drug's Log S ESOL solubility calculation, which is the customary estimation model for analyzing the aqueous solubility of chemical compounds [5]. A boxplot for analyzing lipophilicity was constructed to visualize the drug's lipophilic or hydrophilic tendencies in comparison to the lipophilicities of common lipophilic compounds, ibuprofen, omeprazole, and cholesterol. To analyze the feasibility of the drug binding of the complex, data was collected through UCSF ChimeraX-1.8 for analyzing hydrogen bonding interactions post-docking of their distances measured in Å between hydrogen bond donor and acceptor atoms in hydrogen bonding interactions and distances between hydrogen atoms bonded to the donor and their acceptor atoms involved in residues interacting with the Peginterferon ligand. To assess the strength and attainability of such bonding interactions, one-sample μ tests were

calculated for differences among the mean donor-acceptor and donor-hydrogen to acceptor distances to their respective optimal distances of 3 Å and 2.1 Å.

Results

When replicating 20 simulated models for the optimal docking complex between protein 8AGK and Peginterferon Alfa-2b, the best model computed a binding affinity of -4.003 kcal/mol, demonstrating a moderately strong binding affinity without the consideration for binding stability and strength deduced from the hydrophobic hotspot interactions and hydrogen bonding results. The structure of its protein-ligand complex is pictured below (Figure 5).

Figure 5. Alfa-2b Ligand in Interaction with Residues of Protein 8AGK (Ligand in Green and Binding Residues in Yellow)

An additional visual model was created to highlight the docking position and location of the Peginterferon ligand within a site of the binding pocket of protein 8AGK (Figure 6). The ligand was computed for docking in the center space of Chain A to maintain non-competitive binding among itself and existing ligands, along with avoiding destabilization by distancing itself from hydrophobic hotspots along the ends of the protein's chains. Upon analysis of the interacting residues with the docked Peginterferon ligand in the neurotoxin binding domain, 238 residue atoms were found to be present in the interaction of the drug ligand, consisting of glutamate, lysine, tryptophan, glycine, phenylalanine, aspartate, asparagine, serine, glutamine, tyrosine, isoleucine, and arginine.

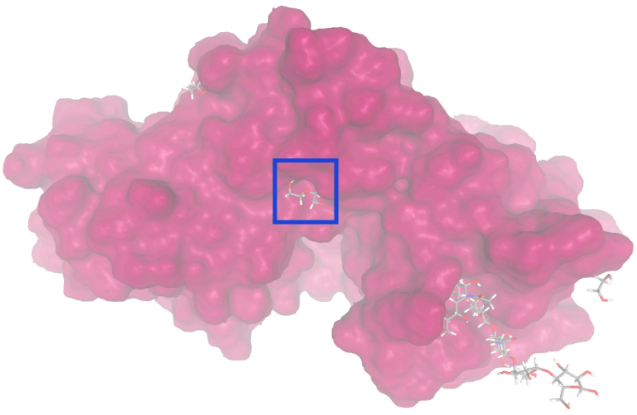

Figure 6. Alfa-2b Ligand Docked Within Binding Pocket of Protein 8AGK

Having performed the one-sample μ tests before docking to analyze the stability of the protein from its hydrophobic interactions, we retrieved P-values of 0.8088 and 0.6304 for the mean respective overlapping and residual distances, as reflected in the following table (Table 1). Such probabilities reveal no statistically significant difference in the mean distance in those of the protein-ligand complex's hydrophobic overlap and residual lengths to the optimal overlap and residual distances.

Table 1. Analysis of Stability and Non-Competitive Binding of Protein 8AGK to Support Docking of Peginterferon Alfa-2b

Distance of Measure	Sample Size, Mean, and Standard Deviation	Optimal Distance	P-Value
Overlapping Between Hydrophobic Regions of Protein-Ligand Residues	Size 26; 0.4104 Å; 0.2168 Å	0.4 Å	0.8088
Interatomic Distance Between Hydrophobic Protein-Ligand Residues	Size 26; 2.3317 Å; 1.7614 Å	2.5 Å	0.6304

In analyzing Alfa-2b's potential for delivery, specifically lipophilicity, in comparison to apparent lipophilic compounds including ibuprofen, omeprazole, and cholesterol, Peginterferon Alfa-2b's computed lipophilic data demonstrates its drug to attribute to hydrophilic tendencies.

The lipophilicity ranges were assessed from a dataset computing the compounds' Log $P_{o/w}$ results. For Peginterferon Alfa-2b, the results were found to be as:

- Log $P_{o/w}$ (iLOGP) at 1.36
- Log $P_{o/w}$ (XLOGP3) at -0.60
- Log $P_{o/w}$ (WLOGP) at -0.27
- Log $P_{o/w}$ (MLOGP) at -0.84
- Log $P_{o/w}$ (MLOGP) at -0.84
- Log $P_{o/w}$ (SILICOS-IT) at -0.72
- Consensus Log $P_{o/w}$ at -0.21

This aligns in correspondence to its customary therapeutic dosage for Hepatitis B where hydrophilicity is desired for eased renal excretion, thus comprising a range mostly below 0 on the Log $P_{o/w}$ scale (Figure 7).

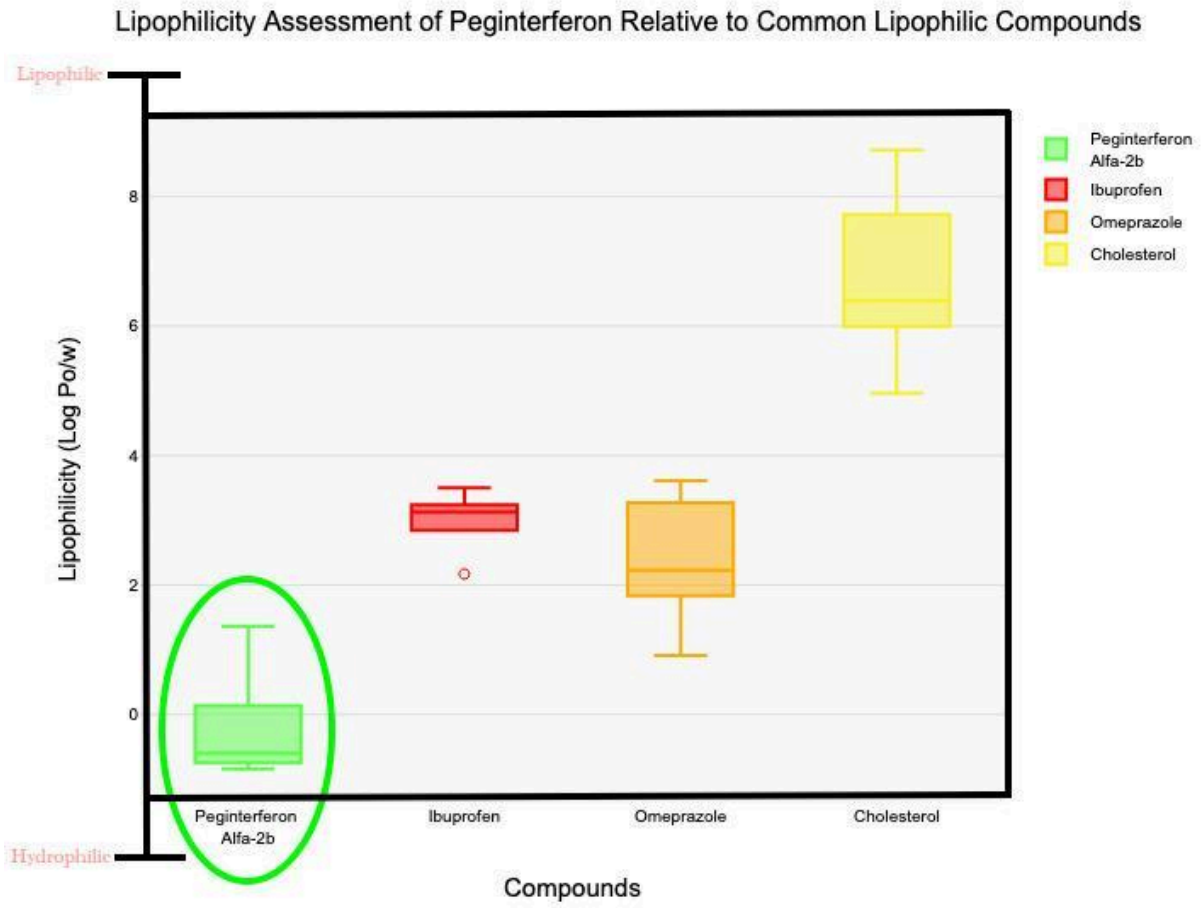

Figure 7. Evaluation of the Lipophilicity of Peginterferon Alfa-2b to Common Compounds with Lipophilic Tendencies

For Alfa-2b's solubility, the computed linear regression predicted a high water solubility of 0.474 mol/L from the initial calculation of 0.06 mol/L from Log S ESOL's software, depicting high bioavailability and swift absorption into the bloodstream after administering the drug (Figure 8).

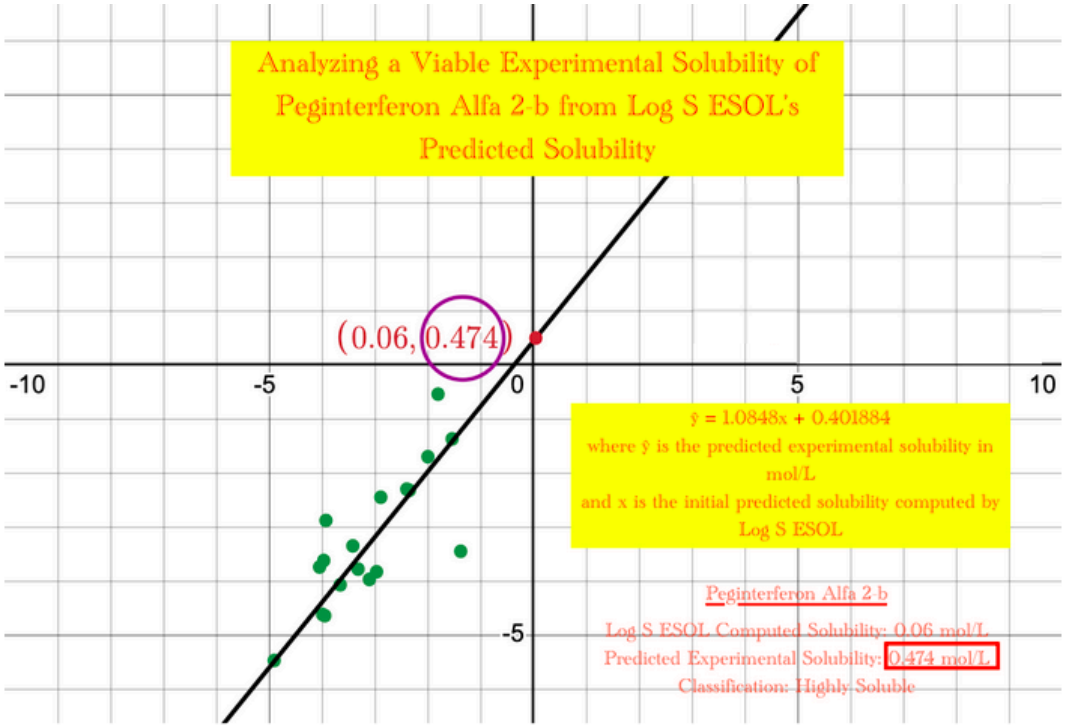

Figure 8. Analysis of a Viable Experimental Solubility of Peginterferon Alfa-2b from Log S ESOL's Predicted Solubility

SwissADME further assessed Alfa-2b's pharmacokinetics (Figure 9). Alfa-2b was seen to resonate with a high GI absorption and a skin permeation of -7.45 cm/s using the computation received from the Log K_p equation. Moreover, Alfa-2b passed the Lipinski (Pfizer) filter for drug-likeness and had a probability of 0.55 from the Abbott Bioavailability Score. Regarding Peginterferon Alfa-2b's medicinal chemistry, 0 alerts were found in its structure from the PAINS and Brenk filters, ensuring the drug is compliant when involved within biological assays. Additionally, Alfa-2b's synthetic accessibility was calculated at 1.94 on a scale of 1 to 10.

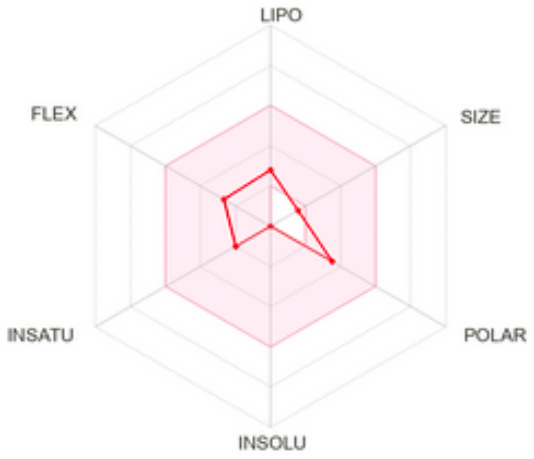

Figure 9. Physicochemical Space Schematic for Oral Bioavailability of Peginterferon Alfa-2b

From performing one-sample μ tests to assess the strength and viability in the geometry of the complex from its hydrogen bonding interactions, we retrieved P-values of 0.3807 and 0.2045 for the mean respective donor-acceptor (D.A) and donor-hydrogen-acceptor (D.H.A) distances as shown below (Table 2). Such probabilities reveal no statistically significant difference in the mean distance in those of the protein-ligand complex's D.A and D.H.A lengths to the optimal distances.

Table 2. Analysis of Strength and Viability of Alfa-2b Binding to Protein 8AGK in Bonding Interactions

Distance of Measure	Sample Size, Mean, and Standard Deviation	Optimal Distance	P-Value
Hydrogen Bonding from Donor Atom to Acceptor Atom	Size 23; 3.0968 Å; 0.5190 Å	3 Å	0.3807
Hydrogen Bonding from Hydrogen Atom of Donor to Acceptor Atom	Size 23; 2.2633 Å; 0.5989 Å	2.1 Å	0.2045

Discussion

From the optimal ligand produced, its position in relative bonding to the protein's already attached PEG and ethylene glycol (EDO) provides continued stability for the binding of the drug of interest, Peginterferon Alfa-2b, as enforced by EDO and synergistic compatibility allowing Peginterferon to coexist amongst the PEG ligands as the PEG molecules amplify the half-life and stability of the Peginterferon when taking effect on the neurotoxin [4]. With heavy interaction in docking among glutamate, lysine, tryptophan, glycine, phenylalanine, aspartate, asparagine, serine, glutamine, tyrosine, isoleucine, and arginine, Peginterferon Alfa-2b carries potential to effectively block the binding site as such binding residues possess adequately strong electrostatic attractions to enhance the binding of the drug ligand and restrict external molecules from also attaching. Hence, GD1a gangliosides would be heavily suppressed in their binding ability to pathogens possessing neurotoxin binding domain proteins like 8AGK as seen in *Clostridium botulinum*, thus restricting such pathogens from instigating the autoimmune response in the peripheral nervous system.

Its binding affinity of -4.003 kcal/mol illustrates its moderate ability to bind strongly enough to attach for an adequate amount of time to elicit a therapeutic response, allowing for the Peginterferon to effectively administer itself when complexed to the cell binding domain of *Clostridium botulinum*. The P-values obtained for testing protein stability with hydrophobic interactions, measuring 0.8088 for hydrophobic overlapping and 0.6304 for hydrophobic hotspot interatomic distances, indicate the model suffices as a favorable structure in stability for binding and upholding non-competitive binding among the hydrophobic hotspots of pre-existing ligands formed from small molecule inhibitors to those of the docked Alfa-2b ligand [6]. Thus, the complex can exhibit an augmented frequency in Van der Waals interactions while limiting further steric clashes from arising post-docking.

Alfa-2b's high GI absorption and negative skin permeation signals an efficient administration of Alfa-2b when absorbed in the gastrointestinal tract, but a limited ability to permeate the skin transdermally. In addition, Alfa-2b passing the Lipinski filter and moderate probability resulting from the Abbott Bioavailability Score affirms a favorable oral administration and is a viable compound for therapeutic use as a drug from its Lipinski assessment [7]. As Alfa-2b did not have any alerts when simulated through the PAINS and Brenk filters, this allows researchers to feasibly implement the drug ligand *in vivo* to measure potency in real time after administration when assessing its therapeutic effects against GBS. The Peginterferon's low synthetic accessibility allows the drug to be easily synthesized for mass replication in the manufacturing process, should this drug be utilized at a mainstream scale as a therapeutic agent against GBS. Hence, the positive scope in Peginterferon Alfa-2b's bioavailability allows for it to enter a mainstream path as a customary treatment for GBS if pursued in the future as it accommodates mass replication and quick analysis in laboratories. The ligand's P-values of 0.3807 for D.A lengths and 0.2045 for D.H.A lengths in evaluating the strength and viability of the binding complex displays the model as a favorable outlook for a feasible geometry of the protein-ligand complex to replicate in reality. It maintains a powerful strength in its binding affinity, helping to restrict the neurotoxin from further attaching to GD1a gangliosides.

Despite such results, limitations are to be addressed with the nature of the study, especially regarding the lack of cross-checking such data produced for real-world replication. Simulations of adding Gasteiger charges and hydrogens differ from their actual presence when

being analyzed in their actual particular interactions as such Van Der Waals' behaviors cannot be precisely replicated onto computational docking software, thus possibly skewing the drug's binding affinity. It may exaggerate the strength and stability of the modeled binding complex, but future laboratory studies can be conducted targeting such factors in Peginterferon's abilities to efficiently bind to the neurotoxin's binding domain when assessing the drug's antagonistic abilities to suppress GBS attacking the GD1a gangliosides.

Conclusion

Ultimately, feasibility has been demonstrated repeatedly in the assessment of the application of Peginterferon Alfa-2b to prevent the binding domain of neurotoxin protein 8AGK from accessing GD1a gangliosides, ushering in the likelihood of implementing this drug for further use as a possible mainstream method of treatment in inhibiting the expression of GBS. This viability is deduced from the positive assessment of Peginterferon's favorable pharmacokinetics coupled with its emphasis on stability and strength when attaching to the cell binding domain of *Clostridium botulinum*, preventing the neurotoxin from the ability to attach to GD1a gangliosides. Thus, Peginterferon Alfa-2b serves as a plausible and promising treatment to use in the preliminary course of treatment for patients newly diagnosed with GBS. The computed caliber of Peginterferon's delivery and binding capabilities pose as a viable drug for use to suppress neurotoxins from stimulating furthered production of AGAs, hence supporting the hypothesis as a viable pharmaceutical to inhibit the demyelinating effects of GBS. The current therapy for GBS being antibodies after the onset of the pathogenic transmission within the body minimizes the likelihood of a patient's recovery to restore normalcy. By pursuing alternate treatments with Peginterferon Alfa-2b to prevent such pathogens from infiltrating the glycosphingolipids branching into the central nervous system, neural plasticity is maintained, allowing for a safer and swifter recovery as muscle atrophy and progressive paralysis are avoided.

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