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Neutralization of Botulinum Neurotoxin with chelating ligand EDTA A Computational Model

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Abstract- Being known as 'Miracle Poison', Botulinum Neurotoxin type- A is one of the most naturally occurring toxic substance known to man. The lethal dosage ofLD50 in humans is 1 ng/kg. Anexotoxin produced by Clostridium Botulinum, a commonly occurring encapsulated gram-positive anaerobic bacterium is found in soil. Easy availability of the toxin and its potential in causing catastrophe due to harmful exploitation like bioterrorism makes it a potential threat that needs immediate precautions. With anti-toxins being already available in the market, the study focus on the simulation of a computational models of ligand molecule Ethylenediaminetetraacetate (EDTA) and its action onBotulinumNeurotoxinwhich has shown some impact on neutralizing the toxin protein. The main intention of this work is to understand the affinity of EDTA on the Zinc ion of Botulinumneurotoxin protein, to understand the druglikeness of EDTAwhich chelates the Zinc ion from the protein and prevents the binding of toxin to SNAP- 25 protein at neuromuscular junction. The work is carried out using a Computational model of Botulinum type-A toxin and EDTA molecules.

Keywords- Botulinum type –A, Neurotoxin, Botulism, EDTA, SNAP-25

1. INTRODUCTION

Clostridium Botulinum serotypes vary from A-G. A new serotype found and named as BoNT/X (serotype X) has been added to the C Botulinum family making the serotype count 8 [1].Botulinum neurotoxin serotype A is one of the most commonly knowncausative agent for botulism. It is also widely used to treat medical conditions like muscle stiffness, uncontrolled blinking of eyelids etc. and in the cosmetic industry, especially to treat wrinkles on the skin [2].

Inspite of the advantages of this neurotoxin in treating various disorders (when properly administered), Botulinum when naturally entered into the system through food, wounds, or intestinal colonization and absorption will result in a condition called Botulism [2]. The potential of this neurotoxin in lethalizing the victim makes it one of the most demanding area to be paid attention for the safety, health of the general public and national security. Only 1g of crystalline toxin has the potential to kill 1 million people [3], being1×10^{11} more toxic than Sodium Cyanide[4]. Several nations have taken advantage of this toxic property and have used the same in biological warfare [5]. The United States Federal Select Agent Program has enlisted botulinum neurotoxin under the HHS and USDA Select Agents and Toxins has enlisted Botulinum as one of the toxins that could pose severe threat to lives of humans and animals [6]. In case of an accidental outbreak of Botulinum toxin due to contaminationof food products (canned food, dairyproducts etc.) or in case of a terrorist attack, immediate and effective measures need to be taken to neutralize the toxin.

Several antitoxin exist in the market that can neutralize the Botulinum neurotoxins of serotype A-G.The United States army has successfully developed heptavalent antitoxin which was used during the Botulism E outbreak in Egypt[4]. A group of researchers at Queensland Brain Institute (QBI), the University of Newcastle and the Children's Medical Research Institute have developed a new molecule called Dyngo-4a™ which prevents the intake of toxin into cell by blocking the function of 'dynamin', a protein responsible for the regulation of molecules in and out of the nerve cells [7].

While several compounds exists that could neutralize the Botulinum toxin, we focused on a new approach to tackle the toxin by creating a computational model EDTA molecule with toxin. EDTA is a chelating ligand ($C_{10}H_{16}N_2O_8$) which is under trial in Clinical Medicine for the treatment of cardiovascular diseases [8], lead toxicity, diabetes and much more. While some studies also show the success of "Chelation Therapy" in chronic degenerative diseases like ischemic heart disease, peripheral vascular disease and intermittent claudication, cerebro-vascular degenerative diseases etc., there exist a debate over the use of EDTA in Clinical Medicine [9]. It is also used in the treatment of lead poisoning in the brain [10] and other disease conditions like diabetes [11], Alzheimer's [12] etc.

This work was carried out to understand the mechanism of EDTA (Ethylenediamidetetracetate) on the Zinc ion of Botulinum neurotoxin and the change in the catalytic property before and after the action of EDTA. The presence and absence of Zinc ion in the toxin changes the catalytic property of the toxin [15]. A complete understanding of the drug-likeness, Physiochemical properties, Pharmacokinetics, Medical Chemistry etc. helps in determining the possibility of using EDTA as an effective drug against the botulism.

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2. MECHANISM OF ACTION

Five modes of Botulism has been identified which include foodborne Botulism, wound Botulism, infant botulism, intestinal colonization, iatrogenic botulism[3].Zinc ion is the major catalytic factor in the action of Botulinum Neurotoxin type- A on Synaptosomal Associated Protein (SNAP -25), a segment of SNARE protein group. This prevents the fusion of Acetylcholine with cell membrane. Botulinum toxin (Light chain) cleaves the segment of SNAP-25 and there by inhibit the neurotransmission of Acetylcholine at neuromuscular junction causing muscle paralysis [2].Once the cleaving of SNAP-25 takes place at the nerve terminal it is irreversible or permanent [2].

One of the several ways to block the action of the toxin is to inhibit the catalytic property of the toxin. This will make it harmless by losing its toxic property. Botulinum toxin type - A is a 150 kDa protein, with a heavy chain of 100-kDa and a light chain of 50-kDa, disulfide noncovalent bond, non-toxin hemagglutinin protein and non-toxin non-hemagglutinin protein [3].Light chain's (50-kDa) proteolytic cleavage of the SNARE protein complex prevents the docking of the acetylcholine vesicle on the inner surface of the cellular membrane and results in blockade of vesicle fusion[13] resulting in the flaccid paralysis when the target is muscle tissue [14].

3. METHODOLOGY

Lance et al., in their paper have showed experiments that are favourable and contradictory to the theory of Zinc ion playing a major role in the catalytic activity of Botulinum toxin. Further they showed thatthe molecule rebinds cation and regains catalytic and neuromuscular blocking activity after the removal of Zn^{2+}[15], while other set of experiments have shown that the action of removal of Zn^{2+} form the toxin in irreversible and therefore removal of Zinc could neutralize the Botulinum toxin and prevent the cleaving of SNAP-25.

Since the experimental analysis was carried out, docking is the suitable method to understand the binding properties of Toxin and ligand molecule EDTA in much detailed manner. Different parameters will give different docking results which will help in understanding the docking under variety of environmental conditions. This allows us to choose the docking predictions which is in alignment with the experimental results with their binding energy and other parameters.

For conducting dockinganalysis, we have used the software Hex v8.0 [16]. 5 successful docking models were analysed and the best result out of it was chosen. The Receptor and Ligand was clustered by energy, where the correlation type was skin plus shape. Once the docking was performed, the visualization was carried out using the PyMol software.

PyMOL is an open-source tool to visualize molecules available from (www.pymol.org))[17]. Validation of the model was done by choosing the best fit by analysing the interaction of the ligand and the receptor, where extend of the binding as of the experimental result was cross validated.

4. RESULT

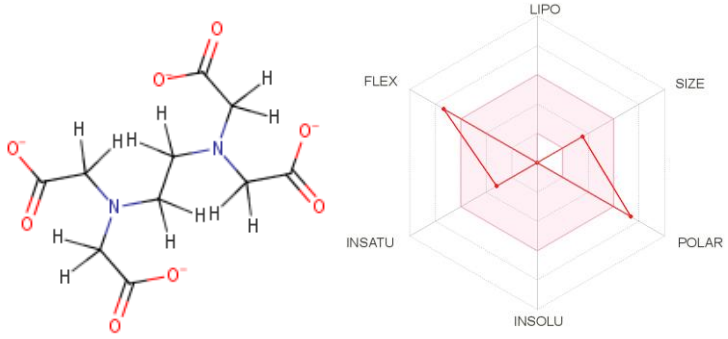
The binding of EDTA to Zinc ion in five different models produced five parameters that are crucial in the picking up of the best model. This five different docking analysis will also help us understand the type of interaction between the Zinc ion of Botulinum and EDTA molecule.The best result out of the five analytical models was picked. The results of the analysis are displayed in the table.

Table 1- Describes the docking calculation result generated out of docking Neurotoxin with EDTA

	Clus	Soluti	Mode	H-bon	Bum	RM	Etotal	Eshap	Eforc	Eair
Model	1	1	0:0	-1	-1	-1.00	-232.	-232.	0.00	0.00

4.1 Admet Properties

SwissADME a free web tool was used to calculate the Pharmacokinetics, drug-likeness, Medical Chemistry of EDTA molecule [18]. The overall ADMET properties assessment result of EDTA is favorable to the use of ETDA in neutralizing Botulinum neurotoxin type A.



(a) (b)

Figure 1. (a). Molecular structure of EDTA, (b). SwissADME Bioavailability Radar image indicating the drug-likeness of EDTA (The pink area represents the optimal range for each properties (lipophilicity: XLOGP3 between −0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 $Å^2$, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds.)
The Bioavailability Radar image predicted EDTA to be not orally bioavailable, because it is too flexible and polar.

Table-2 Physiochemical properties

Formula	C10H12N2O8
Molecular weight	288.21 g/mol
Num. heavy atoms	20
Num. arom. heavy atoms	0
Fraction Csp3	0.60
Num. rotatable bonds	11
Num. H-bond acceptors	10
Num. H-bond donors	0
Molar Refractivity	55.29
TPSA	167.00 Ų

Table- 3Lipophilicity

Log Po/w (iLOGP)	Log Po/w (XLOGP3)	Log Po/w (WLOGP)	Log Po/w (MLOGP)	Log Po/w (SILICOS-IT)	Consensus Log Po/w
0.17	-5.88	-7.41	-1.79	-2.44	-3.47

Table 3 show Partition coefficient between n-octanol and water, iLOGP,XLOGP3,WLOGP,MLOGP,SILICOS-IT are five different freely available predictive models to calculate Lipophilicity.
Three different model (ESOL, Ali et al.,SILICOS-IT) have been used to predict the water solubility of EDTA.

Table-4Water Solubility

Log S (ESOL)		Log S (Ali)		Log S (SILICOS-IT)	
Solubility	1.83e+05 mg/ml ; 6.36e+02 mol/l	Solubility	3.19e+05 mg/ml ; 1.11e+03 mol/l	Solubility	9.77e+03 mg/ml ; 3.39e+01 mol/l
Class	Highly soluble	Class	Highly soluble	Class	Soluble

Table 4 shows all predicted values are the decimal logarithm of the molar solubility in water (log S). SwissADME also provides solubility in mol/l and mg/ml along with qualitative solubility classes

Table-5Pharmacokinetics

GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (skin permeation)
Low	No	Yes	No	No	No	No	No	-12.23 cm/s

The Pharmacokinetics calculation of EDTA molecule shows that the GI absorption is low and there is no Blood-Brain Barrier permeability. EDTA being a substrate to P-Glycoprotein shows poor absorption and bioavailability. Log Kp (skin permeation value) -12.23 show that skin permeation is very low which show that administration through skin also fails in delivering the drug. The molecule also acts as a non-substrate to 5 isoforms of cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) which is crucial the elimination of drug from the body [19]. The overall result obtain signifies that the oral drug administration is not of much use, which leaves the intravenous administration of EDTA the better option.

Table- 5 Druglikeness

Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Yes; 0 violation	No; 1 violation: WLOGP<-0.4	No; 2 violations: Rotors>10, TPSA>140	No; 1 violation: TPSA>131.6	No; 2 violations: XLOGP3<-2, TPSA>150	0.11

The fact that EDTA follow all Five Lipinski rule is a favourable chance for the use of the molecule as a drug.
Table-6 Medicinal Chemistry

PAINS	Brenk	Leadlikeness	Synthetic accessibility
0	0	No; 1 violation: Rotors>7	1.93

In table 6, PAINS, Brenk are two pattern recognition methods allow for identification of potentially problematic fragments.

5. CONCLUSION

When bugs keep evolving into superbugs and new strains of Clostridium toxins are discovered, a reliable method and technique need to be developed that will neutralize all the serotypes of Botulinum with lower cost and high effectiveness satisfying ADME properties, druglikenesswith minimal side effects. With the increase in the computational knowledge and resources, availability of data (structure, sequence, pathway, mechanism of action etc.) more attention must be paid to computational drug discovery and model simulation to understand the interaction of toxic proteins with body and the effectiveness of novel drugs in neutralizing the toxin. Computational approach has proven successful in understanding the threats of Ebola and Zika virus in shortest of time and able to comeup with solutions. The conclusion being, a faster threat needs a faster solution, therefor drug discovery with computational techniques need to be well adopted to make the process efficient.

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