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Research Article

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In silico analysis of protein vamp7 signatures involved in tetanus

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ABSTRACT

TETANUS which is more familiarly known as LOCKJAW is a medical condition characterized by a prolonged contraction of skeletal muscle fibers. The disease is caused by tetanospasmin, a neurotoxin produced by the Gram-positive, obligate anaerobic bacterium Clostridium tetani found mainly in free living form in soil. Infection generally occurs through wound contamination and often involves a cut or deep puncture wound. As the infection progresses, muscle spasms develop in the jaw (thus the name "lockjaw") and elsewhere in the body. The main emphasis of this in silico analysis was to find different genes expressed and proteins formed during the diseased condition in humans and to study the evolutionary relationships between the expressed proteins. Single out the group having a common ancestry so as to find out what common domains these proteins share that are functionally active during the disease and then to analyze these domains to find the motif patterns that are altered and modified in these diseased proteins._Our main aim was to identify these motif patterns and to analysis these to observe notable changes in the patterns of the proteins belonging to the same family so as to find the changes that occur in the structure of the proteins that are otherwise evolutionarily related. These patterns when identified could be used for the purpose of mutation studies, drug designing, pattern recognition and diagnostic purposes. After all the analysis of various proteins involved in TETANUS, we drew the inference that the conserved motif patterns with little variations are found in common domains of proteins VAMP7, VAMP3, VAMP2, hence this is the pattern that is altered and plays some role in the whole disease process. This could further be justified by doing various mutation studies.

INTRODUCTION

Tetanus is a medical condition characterized by a prolonged contraction of skeletal muscle fibers. The primary symptoms are caused by tetanospasmin, a neurotoxin produced by the Gram-positive, obligate anaerobic bacterium *Clostridium tetani*. We have already studied QSAR Studies of 6-aryl- 6*H*-pyrrolo [3, 4-d] pyridazine analogues as highaffinity ligands of the α2δ subunit of voltage-gated calcium channels[1].

In humans Tetanospasmin binds to motor nerves that control muscles, enters the axons (filaments that extend from nerve cells), and travels in the axon until it reaches the body of the motor nerve in the spinal cord or brainstem [2,3]. Then the toxin migrates into the synapse, where it binds to presynaptic nerve terminals and inhibits or stops the release of certain inhibitory neurotransmitters (glycine and gamma-amino butyric acid) [4]. Because the motor nerve

has no inhibitory signals from other nerves, the chemical signal to the motor nerve of the muscle intensifies, causing the muscle to tighten up in a huge continuous contraction or spasm.

The hallmark feature of tetanus is muscle rigidity and spasms. Irritability, muscle cramps, sore muscles, weakness, or difficulty swallowing are commonly seen [5]. Facial muscles are often affected first. Trismus or lockjaw is most common. This condition results from spasms of the jaw muscles that are responsible for chewing. A sardonicsmile -- medically termed *risussardonicus* -- is a characteristic feature that results from facial muscle spasms[6,7].

The clostridial neurotoxin-insensitive soluble *N*-ethylmaleimidesensitivefactor attachment protein (SNAP) receptors, tetanus neurotoxin-insensitive (TI)-vesicle-associated membrane protein(VAMP)/VAMP7, SNAP23, and syntaxin 3 have recently been implicated in transport of exocytotic vesicles to the apical plasma membrane of epithelial cells. This pathway had been shown previously to be insensitive to tetanus neurotoxin and botulinum neurotoxin F. TI-VAMP/VAMP7 is also a good candidate to be implicated in an exocytotic pathway involved in neurite outgrowth because tetanus neurotoxin does not inhibit this process in conditions in which it abolishes neurotransmitter release[8,9,10].

TI-VAMP/VAMP7 (Tetanus neurotoxin insensitive-vesicleassociated membrane protein) interacts with its t SNARE partners, particularly plasmalemmalsyntaxins, to mediate membrane fusion and with several regulatory proteins especially via its amino-terminal regulatory.Partners include AP-3, Hrb/(Human immunodeficiency virus Rev binding) protein, and Varp (Vps9 domain and ankyrin repeats containing protein)and regulate TI-VAMP's function and targeting. TI-VAMP is involved both in secretory and endocytic pathways[11-14]. Which mediate neurite outgrowth and synaptic transmission, plasma membrane remodeling and lysosomal secretion[15,16].

VAMP7 and the lysosomal glycoproteinLamp1 extensively colocalize in vesicles present throughout the soma and neurite outgrowths of primary sympathetic neurons. [17-19].

EXPERIMENTAL SECTION

Retrieving expressed genes and proteins:
In order to find the genes expressed and proteins formed during the diseased condition of tetanus, we used **GeneCards** database which is being managed by Human Genome Center and Weizmann Institute of Science. GeneCards provides gene-centric information, automatically mined and integrated from a myriad of data sources, resulting in a web-based card for each of the tens of thousands of human gene entries. It contains large amount of information collected from different databases about different genes and proteins involved in various diseases.

We typed tetanus in the Google search text box and, the result displayed consisted of **111 genes out of which 108 encoded proteins.** Then various information and function of different proteins were collected and stored.

Finding Proteins with common ancestry:
Phylogenetic Analysis was done on all the proteins using SDSC Workbench**,** where a new session was created and then all the proteins were uploaded for analysis. Then by using the CLUSTALW tool a phylogentic tree of the proteins was constructed and studied using BLOSUM 80 matrix. Then from the studies done, the proteins with common ancestry were singled out to be used for further analysis.

Finding common domains in selected proteins:
PRODOM (protein domain database)that has been formed by automatically clustering segments. Source protein sequences are non-fragmentary sequences derived from UniProtKB (Swiss-Prot and TrEMBL databases). It is now maintained by the PRABI (bioinformatics center of Rhone-Alpes). The ProDom database consists of domain family entries. Each entry provides a multiple sequence alignment of homologous domains and a family consensus sequence. **PRODOM** was used to find all the functional domains in humans of the selected proteins. Then these domains were tabulated and analyzed to find the proteins that have some common domains, so that motif analysis of these domains could be done to carry forward our study.

Finding conserved motif pattern:
PMotif & Finger Print SCAN databases consisting of motifs present in different domains of various proteins were used to find the motifs present in the common domains of the selected proteins. Then proteins with common

conserved motif patterns were found and analyzed to study the variation caused in the pattern caused due to disease intervention.

3-D visualizations of the patterns:

Discovery Studio is a software suite of life science molecular design solutions for computational chemists and computational biologists. Discovery Studio makes it easier to examine the properties of large and small molecules, study systems, identify leads and optimize candidates. Discovery studio was used for creating 3-D structure of the conserved motif pattern for helping various mutation and drug designing studies.

RESULTS AND DISCUSSION

On searching Gene Card for proteins involved in TETANUS, we found **111 Genes** were involved. Of which **1 gene locus** and **2 proteins** without function and of the remaining **108, VAMP7** had the highest score followed by **VAMP3 and VAMP2.**

After the phylogenetic analysis it was discovered that **thirteen** proteins had a common ancestor. Phylogenetic tree of the same is given in **Fig.1** Which tells of about the relationship of different proteins with each other, such as which ones are closely related and which ones are distantly related. **Their properties such** genes from which they are expressed and their score are given **in Table.1**

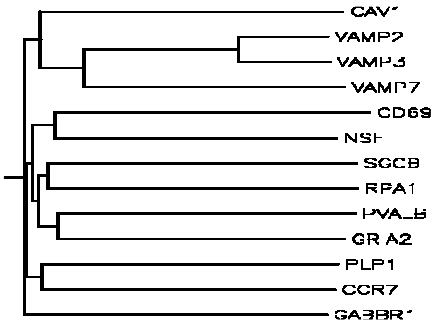

Fig.1: Phylogenetic tree of thirteen proteins with same ancestor

Then the domain analysis by PRODOM of these proteins stated that only **eight** of them shared common domains, which were further considered for further analysis to find various conserved motif patterns .these **eight Proteins domains** are **VAMP7, VAMP3, VAMP2,CCR7, PLP1, GABBR1, CD69, GRIA2 .**

Then Pmotif and fingerprint SCAN were used to find different conserved motif patterns in the common domain. And on analysis of these domains it was found that out of these 8 proteins only 3 VAMP7, VAMP3 and VAMP2 had common conserved motif patterns with slight variations.

The patterns of which are stated below and their 3-D structures of the domains with these conserved patterns are given in **Fig.2, Fig.3 and Fig.4 respectively,** which tells us about how the different amino acid are arranged in the 3-D environment, how they are arranged whether in form of helix or sheets and also tells the location and stretch of the conserved motif under study.

VAMP7QAQVDELKGIMVRNIDLVAQ
VAMP3QNQVDEVVDIMRVNVDKVLE
VAMP2QAQVDEVVDIMRVNVDKVLE

So as we found that the motifs of VAMP2 and VAMP3 were almost similar but that of VAMP7 had major variation so it was concluded that the specific signature of VAMP7 was involved in tetanus. Further analysis of the above given **DOMAIN STRUCTURE** of **VAMP7** can be done for **mutation studies** that is we can study the amino acid sequences and patterns in VAMP7 domains to find out the residues and positions that have been changed over a period of time, which in turn has resulted in altering of the functionality of that particular domain of VAMP7.

Consequently this domain structure can also be used for drug designing to find new, more effective and safer drug.

CONCLUSION

Tetanus is an infectious disease that affects human nervous system, when infected by the gram +ve bacteria Clostridium tetani that produces neurotoxin tetanospasmin.

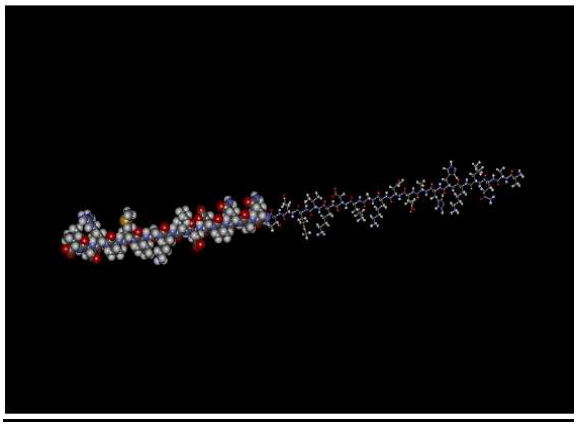

Fig.2: Structure of VAMP7 Domain with altered conserved motif.

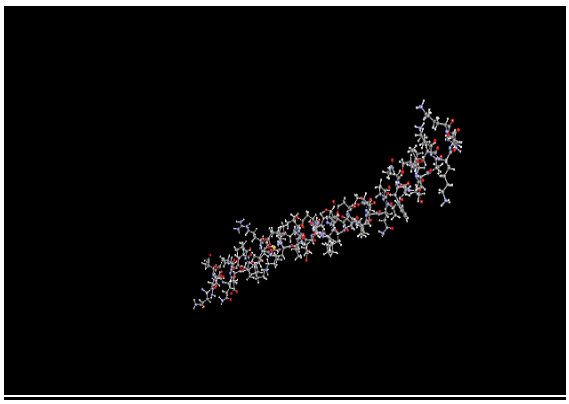

Fig.3: Domain structure of VAMP3

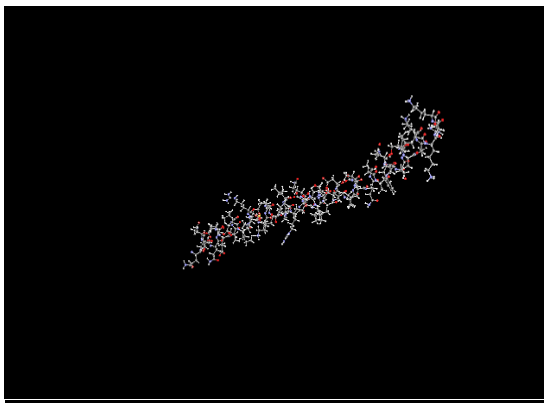

Fig.4: Domain structure of VAMP2

During the span of our studies we discovered that proteins **VAMP7, VAMP3 and VAMP2** are affected in tetanus by tetanospasmin. Out of which conserved motif pattern of **VAMP7** was discovered to be altered, suggesting that it might be affected by the neurotoxin.

So we suggest that this altered motif pattern should be further analyzed for **mutation studies** and for drug designing studies to find a more potential safe and effective drug.

Table.1: List of 13 proteins with same ancestors.

S. No.	GENE	PROTEIN	SCORE
1	VAMP7	Vesicle-associated membrane protein 7	2.32
2	VAMP3	Vesicle-associated membrane protein 3	0.48
3	VAMP2	Vesicle-associated membrane protein 2	0.48
4	CD69	CD69 molecule	0.48
5	CAV1	caveolin 1, caveolae protein	0.48
6	NSF	N-ethylmaleimide-sensitive factor	0.48
7	SGCB	sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)	0.48
8	RPA1	replication protein A1	0.48
9	GRIA2	glutamate receptor, ionotropic, AMPA 2	0.48
10	PLP1	proteolipid protein 1	0.48
11	PVALB	parvalbumin	0.48
12	GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	0.48
13	CCR7	chemokine (C-C motif) receptor 7	0.48

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List of Abbreviations

S.NO.	ABBREVATION	FULL FORMS
1	VAMP7	Vesicle-associated membrane protein 7
2	VAMP3	Vesicle-associated membrane protein 3
3	VAMP2	Vesicle-associated membrane protein 2
4	CD69	CD 69 molecule
5	CAV1	caveolin 1, caveolae protein
6	NSF	N-ethylmaleimide-sensitive factor
7	SGCB	sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)
8	RPA1	replication protein A1
9	GRIA2	glutamate receptor, ionotropic, AMPA 2
10	PLP1	proteolipid protein 1
11	PVALB	parvalbumin
12	GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1
13	CCR7	chemokine (C-C motif) receptor 7