Uses of botulinum toxin in ophthalmology

An Essay
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List of Abbreviations

ACH	Acetyl Choline
ASI	Anterior Segment Ischemia
BTX-A	Botulinum toxin type A
EMG	Electromyography
KDa	Kilo Dalton
Mm	Millimeter
NaCl	Sodium Chloride
SNAP-25	Synaptosomal Associated Protein
SNARE	Synaptosomal Associated Protein Receptor
TNO	Toegepast Natuurwetenschappelijk Onderzoek (*Dutch Organization for Applied Scientific Research*)
U	Units
VAMP	Vesicle Associated Membrane Protien

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Introduction

Botulinum toxin A is the most powerful member of a group of neurotoxins that are produced by clostridium botulinum bacteria. It is a neuromuscular blocking agent that acts by binding to the nerve terminals preventing the release of acetylcholine leading to functional muscle denervation. It acts on striated and smooth muscles as well as parasympathetic and cholinergic postganglionic sympathetic neurons,(1).

Botulinum toxin type A is widely used in medical practice. It has been first used to treat cases of strabismus. Now its indications had increased to include dystonic movement disorders, strabismus, nystagmus, headache syndromes such as migraine, lacrimal hypersecretion syndromes, eyelid retraction, spastic entropion, compressive optic neuropathy, treatment of dynamic facial lines e.g. frown lines and laughter lines for facial rejuvenation, correct asymmetry of the face,and reshape facial features without surgery. But the application of botulinum toxin for strabismus and cosmetic management are the most common uses(2).

Botox and Dysport are two available commercial forms of botulinum toxin type A. Both are provided in a lyophilized form and must be reconstituted before use,(3).

There are some precautions to be taken when using botulinum toxin type A. Recovery occurs over 3 to 4 months from nerve terminal sprouting and regeneration of inactivated proteins necessary for degranualtion of acetylcholine vesicles(4).

There are numbers of potential complications with the use of all clinical preparations of botulinum toxin. Most of these result from its ability to chemodenervate striated muscles. However, almost all of these side effects are mild and transient, and the incidence is generally rather low, and rarely significant enough to make the patient discontinue treatment. The most common complications are ptosis, blurred vision, dry eyes , tearing ,ectropion, keratitis, diplopia , entropion, facial weakness and weakness of lip elevation,(5).

Aim of the work

This work aims to review the literature about the most common types of botulinum toxins, their common uses in ophthalmology, side effects and the most important precautions should be taken while used.

Chapter 1

Informations on botulinum toxin

Pharmacology .
Mechanism of action .
Microscopic changes at neuromuscular junction after Botox injection.
Commercial preparations .
Reconstitution .
Storage of reconstituted toxin .

Botulinum toxin is one of the most potent toxins that blocks the release of acetylcholine at the neuromuscular junction. The use of botulinum toxin has spread through different subspecialties1-6. It is probably one of the most investigated usages wherever there is the need to block muscular activity (6).

It is produced by clostridium botulinum bacteria which are large rod shaped, anaerobic and spore-forming gram positive organisms(fig.1). It was first identified as a causative agent in food poisoning more than 100 years agoin 1895 in Belgium by Professor ***Emile Pierre Van Ermengem*** (7) .

Figure 1.Clostridium botulinum bacteria (8).

Pharmacology:

Botulinum neurotoxins represent some of the most toxic, naturally occurring substances. They include eight immunologically distinguishable exotoxins (A, B, C alpha, C beta, D, E, F and G). Types A, B and E are commonly associated with toxicity to humans (9).

They are structurally similar and are all capable of interfering with acetyl choline release. Yet, they vary in their biosynthesis, size and cellular mechanism of action. All the serotypes bind to the same receptor but target different proteins and enzymes within the synapse. These specific differences and species sensitivity suggest that each serotype has its own unique pharmacological entity (10).

The different serotypes vary with respect to their potency and duration of their effect and consequently, they vary in their clinical applications. Type A is the most powerful of the eight types and its effects are the longest lasting. It is around twenty times more toxic than type B (11).

In fact, botulinum toxin type A was the first to be developed for clinical use and now it is commonly used. Types B and F have also shown beneficial effects in humans and a commercial preparation of type B has recently become available. The other serotypes are inadequately studied at this time but it is anticipated to find out clinical applications for them in the future (12).

All of these neurotoxins have high content of hydrophobic amino acids residues that may be relevant to the membrane binding internalization of toxin. They exist in their native forms as large protein complexes (fig.2). They consist of neurotoxin (fig.3) almost 150 kilo Dalton “KDa” and one or more nontoxic proteins that may or may not have hemagglutinin activity. For example, botulinum

toxin type A “BTX-A” binds with nontoxic proteins to form a complex of a total molecular weight of approximately 900KDa (13).

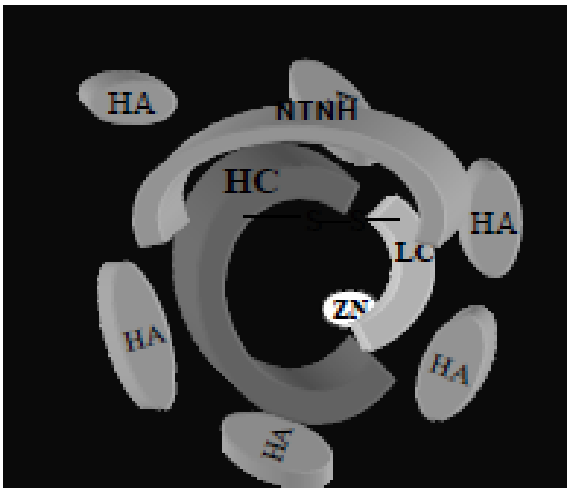

Figure 2.The structure of botulinum toxin complex. HC:heavy chain, LC:light chain, HA:hemagglutin ptn, NTNH:non-hemagglutin ptn , ZN: zinc (14)

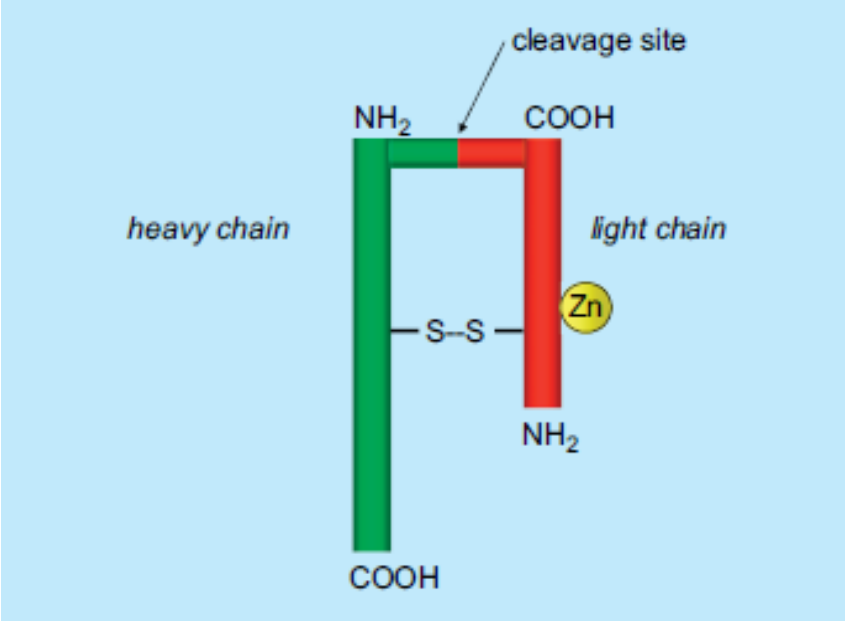

Figure 3. Structure of botulinum neurotoxin molecule. The native molecule is cleaved to form a light and heavy chain, joined by disulfide bond(s-s), COOH: C-terminal fragment, NH2: N-terminal fragment (15).

The non toxic proteins provide protection to the neurotoxin subunit which when ingested orally must endure the harsh environment of the gut before being absorbed. The larger the molecular size of the nontoxic component, the greater the protection afforded to the neurotoxin from gastric acidity and proteases (16). In therapeutic preparations, purified forms of these botulinum toxin complexes help to stabilize the activity of the neurotoxin during its production and to potentially reduce the undesired spread of the toxin to the adjoining muscles when injected (17).

The neurotoxin component is synthesized as a single-chain polypeptide with a molecular weight of approximately 150 KDa . The neurotoxin must be proteolytically cleaved or "nicked" by endogenous bacterial proteases into an active dichain form (fig.4). The nicking occurs at a peptide bond approximately one third the length of the polypeptide from the N-terminal. The resultant dichain form consists of a light chain (50 KDa) and a heavy chain (100 KDa) linked by disulfide bond (18).

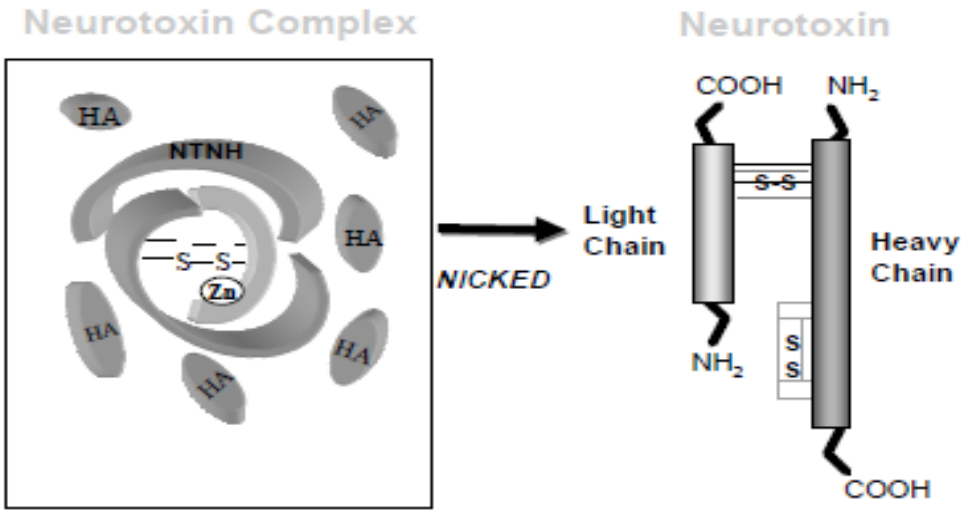

Figure 4.The neurotoxin complex nicking. The native molecule is cleaved to form a light and heavy chain, joined by disulfide bond(s-s), COOH: C-terminal fragment, NH2: N-terminal fragment.

The light chain contains a zinc-binding motif that has a specific intracellular proteolytic activity and associated with neurotoxicity (19).

The heavy chain contains 2 functional domains:

- N-terminal fragment associated with translocation of the light chain into the cytosol (18).
- C-terminal fragment involved with binding to neuronal receptor sites. It is the most dissimilar in amino acid sequence, contributing to the unique specificity of each serotype for its own binding sites. This is also the region associated with the development of neutralizing or inactivating antibodies. As a result, neutralizing antibodies developed against one serotype have not been reported to block the biological activity of another serotype (20).