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Investigating the dual nature of *Clostridium botulinum*: pathogenic mechanisms and therapeutic potentials

Kosar Soleymanpour[1], Elham Zeinalzadeh[2], Khudaverdi Ganbarov[3], Mohammad Asgharzadeh[4], Hossein Samadi Kafil[5]

[1]Department of Cellular and Molecular Biology, Ta.C., Islamic Azad University, Tabriz, Iran;
[2]Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran;
[3]Research Laboratory of Microbiology and Virology, Baku State University, Baku, Republic of Azerbaijan;
[4]Biotechnology Research center, Faculty of Para Medicine, Tabriz University of Medical Sciences, Tabriz, Iran;
[5]Drug Applied Research center, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

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SUMMARY

Background: Clostridium botulinum (*C. botulinum*) is known for producing one of the strongest neurotoxins, namely botulinum neurotoxin (BoNT). This toxin is responsible for causing botulism, which is considered a severe neurological disease.
Objectives: This comprehensive narrative review highlights the dual nature of *C. botulinum* as both a serious health threat and a groundbreaking therapeutic agent. Methods: A comprehensive literature search was conducted in Google Scholar, PubMed/Medline, and Scopus (1971–2025, 2020–2025 literary focus) using keywords related to botulism pathogenesis, clinical features, and therapeutic approaches.
Results: Key findings indicate that, despite advancements in botulism management and reduced mortality due to heptavalent botulinum antitoxin (HBAT) and botulism immune globulin intravenous (BIG-IV) antitoxins, diagnostic challenges and bacterial spore resistance, particularly in group I, remain persistent. On the other hand, the discovery of serotype BoNT/X with lower toxicity in vertebrates and unique capabilities has opened new horizons in the design of safer treatments.
Conclusions: C. botulinum represents a remarkable paradox in biology, an organism capable of producing one of the deadliest toxins known, yet simultaneously a cornerstone in modern therapeutic innovation. In fact, this toxin's dual nature is based on its selective targeting of BoNTs. In this regard, the systemic entry and distribution of this poison in the body - or, in other words, intoxication with it - poses a life-threatening aspect. On the other hand, the use of this bacterium's toxin for therapeutic and medicinal purposes is progressing due to its topical and controlled administration and entry into the body make it a targeted and effective therapeutic tool in the field of medicine. A better and more precise understanding of the mechanisms of action and, if necessary, future structural and engineered modifications in the toxin could lead to the emergence of a new generation of BoNTs that hold promise for the development of esthetic and therapeutic applications in medicine.

Keywords: Clostridium botulinum, botulinum neurotoxin, botulism, botulinum antitoxin.

Corresponding author
Hossein Samadi Kafil
E-mail: Kafilhs@tbzmed.ac.ir

INTRODUCTION

Zoonotic diseases refer to diseases that are common to both humans and animals, transmitted from animals to humans, and account for about 75% of infectious diseases identified in the past decade [1]. Botulism is a zoonosis with low

prevalence but a high mortality rate, which has become one of the high-risk diseases due to its unusual clinical manifestations and difficult diagnosis. This disease is a neuromuscular disorder that was first discussed in the 10th century as one of the foodborne illnesses, especially in young children. Over time, it became clear that this disease can affect individuals of different ages [2]. Justinus Kerner was the first person to describe the precise and complete symptoms of botulism between the years 1817 and 1822 and stated that a biological toxin is the main cause of this disease [3]. The toxin produced by the bacterium *Clostridium botulinum* (*C. botulinum*), known as botulinum neurotoxin (BoNT), is the cause of the neurological paralysis disease botulism. It is also increasingly recognized for its therapeutic applications in treating various medical conditions characterized by overactive or spastic muscles. The effectiveness of BoNT in reducing muscle contractions not only addresses medical disorders but also meets aesthetic needs, such as reducing facial wrinkles [4]. In recent years, despite significant advancements in understanding *C. botulinum* in the fields of microbiology and neurotoxins, scientific understanding of its duality as both a pathogen and a source of therapeutic potential represents a significant knowledge gap. Most previous studies have generally focused on the descriptive aspects of pathogenesis and conventional treatments and have paid less attention to examining the dual nature of the toxin produced by this organism. Therefore, the aim of the present study is to provide a comprehensive and analytical overview that examines the combination of pathogenicity, structural and therapeutic aspects, and their interrelationship. A deep understanding of molecular processes can be instrumental in modifying the biological structure and function of existing neurotoxins, as well as in re-engineering new-generation neurotoxins with enhanced safety and selectivity, thereby shaping the future of neurological treatments.

METHODS

A comprehensive search was conducted using scientific databases Google Scholar, PubMed/Medline and Scopus for English publications published from 1997 to 2025, focusing on the latest five years (2020–2025) to assure content updates. The literature search was conducted using the following keywords combined with Boolean operators: ("*Clostridium botulinum*" OR "botulinum neurotoxin" OR BoNT OR BoNTs) AND (botulism OR "botulism pathogenesis") AND ("proteolytic strains" OR "non-proteolytic strains") AND ("botulinum antitoxin" OR "monoclonal antibodies" OR "botulism treatment") AND (neuromodulator OR "therapeutic applications" OR "therapeutic use"). To comprehensively cover various aspects, various types of studies were used, including reviews, laboratory research, book-based and educational resources, epidemiological studies, case reports, randomized trials, and clinical guidelines. We excluded studies that addressed non-botulinum toxins or unrelated pathogens, articles in non-English languages, and those lacking valid data, duplicates, or outdated information. The EndNote® software was used for resource man agement and the removal of duplicate articles. The figure included in this study was independently created by the author using Microsoft PowerPoint, with conceptual inspiration drawn from previously published scientific literature. The present study, with a focus on transparency in reporting, citation of primary sources, and the latest credible findings, has adhered to the general principles of research ethics.

CLOSTRIDIUM BOTULINUM AND ITS NEUROTOXINS

Clostridium botulinum is a Gram-positive, saprophytic, and obligate anaerobic bacillus, a member of the largest bacterial genus named Clostridium, the family Clostridiaceae, and belonging to the phylum Firmicutes. *C. botulinum* is motile due to its peritrichous flagella. It is also classified as a catalase-negative and oxidase-negative bacterium. This bacterium produces oval-shaped endospores and exotoxin, which are typically found in freshwater, marine sediments, and soil [4, 5]. The spores produced by *C. botulinum*, due to the presence of a thick protein coat surrounding their core cells, are resistant to radiation, oxygen, heat, and various chemicals. *C. botulinum* is classified into groups I to IV based on metabolism, pathogenesis, 16S rRNA sequences, and amplified fragment length polymorphism (AFLP) analysis [6]. Group I includes proteolytic strains that produce toxins A, B, and F. Group II includes non-proteolytic strains producing toxins B, E, and F. Both groups,

I and II, are considered pathogenic strains for humans. Group III includes strains that produce toxins C or D, which have been identified exclusively in animals and play a role in animal botulism. Finally, group IV includes strains that produce toxin G [7]. Each of the produced toxins are serologically different, and the identification of bacterial strains and the subsequent use of antitoxins are based on determining the type of toxin [8]. The proteolytic group produces heat-resistant spores, and their optimal growth temperature is 35 degrees Celsius. In contrast, the non-proteolytic group produces spores that are sensitive to high temperatures, with a minimum optimal growth temperature of 4 degrees Celsius. In most cases, type A and B toxins from group I are associated with infant botulism. Although it can also be caused by type E and F toxins of group II [9]. BoNT is the strongest neurotoxin and a zinc metalloprotease that inhibits the exocytosis of cholinergic neurotransmitters at the neuromuscular junctions (NMJ) and ultimately leads to flaccid paralysis. BoNT includes serotypes A to G and, based on its amino acid sequence, encompasses more than 40 subgroups [10, 11] (*Table 1*). Genetic sequencing of *C. botulinum* strain 111 led to the discovery of a new serotype called BoNT/X. This strain also produces BoNT/B2, but the gene producing BoNT/X is located on the bacterial chromosome, while the gene producing BoNT/B2 is located on a plasmid. Based on the findings, the protein structure of BoNT/X has the least similarity with other serotypes (A-G). It is also the only toxin that, in addition to being able to cleave vesicle-associated membrane proteins (VAMPs) 1, 2, and 3 like other serotypes, cleaves VAMP2 in a new region (between the amino acids arginine-66 (Arg66) and alanine-67 (Ala67)). Additionally, it is the first and only toxin capable of degrading new substrates (VAMP4, VAMP5, Ykt6), recognizing the peptide bond between the lysine–serine (Lys-Ser) in VAMP4 and Ykt6, and between the arginine–serine (Arg-Ser) in VAMP5 as the cleavage site. Although BoNT/X exhibits high enzymatic activity, according to conducted experiments, it seems to have significantly lower toxicity to vertebrates, including humans, compared to other serotypes. Therefore, it can be considered an ideal candidate and a potential target for novel therapies [12-14]. BoNT is a polypeptide with a weight of 150 kilodaltons, in which a 100-kilodalton heavy chain (HC) is linked to a 50-kilodalton light chain (LC) through a disulfide bond. This toxin has three distinct regions in its structure: N, middle, and C. Among these, the C region binds to the presynaptic membrane, the middle region facilitates the entry of LC into the cytosol, and finally, the N region acts as a polypeptidase [15]. BoNT also contains a non-toxic component called neurotoxin-associated proteins (NAPs), which are themselves composed of hemagglutinin (HA) proteins and non-toxic non-hemagglutinin

Table 1 - Classification of *C. botulinum* and its neurotoxins.

BoNT serotype	*Group*	*Bacterial metabolic type*	*Subgroup of toxins*	*Botulism-related items*
BoNT/A	Group I	Proteolytic	A1, A2, A3, A4, A5, A6, A7, A8	Human
BoNT/B	Group I	Proteolytic	B1, B2, B3, B5, B6, B7	Human
	Group II	Non-proteolytic	B4	
BoNT/C	Group III	Proteolytic	C, CD	Animals (birds)
BoNT/D	Group III	Proteolytic	D, DC	Animals (cattle)
BoNT/E	Group II	Non-proteolytic	E1, E2, E3, E6, E7, E8, E9, E10, E11	Human
BoNT/F	Group I	Proteolytic	F1, F2, F3, F4, F5	Human
	Group II	Non-proteolytic	F6	
BoNT/G	Group IV	Proteolytic	G	Lack of identification in humans or animals. (Environmentally isolated only)

(NTNH) proteins. Among these, the presence of certain specific antigenic proteins, which are a subset of NTNH, leads to the formation of antibodies in the treatment of BoNT [16]. So far, the lethal dose of BoNT has not been precisely determined; however, it is referred to as the most toxic toxin. It is estimated that in a 70 kg man, the lethal dose (LD) of BoNT type A would be 0.09 to 0.15 micrograms if injected intravenously, 0.8 to 0.9 micrograms if inhaled, and 70 micrograms if taken orally. However, according to other studies, lower doses have also been reported [17]. In summary, lethality and immunotherapy can be considered the dual biological model of this organism, caused by the molecular diversity, exceptional properties, and potency of its toxins. Continuous research in the field of new serotypes and the discovery of unique features such as BoNT/X not only leads to a redefinition of neuroinhibitory mechanisms but also has the potential to shape the future of precise and targeted neurotherapies and open a new path for neuroregenerative medicine.

SYMPTOMS AND TYPES OF BOTULISM

Botulism is considered a rare and very serious disease that manifests as cranial nerve paralysis. This occurs through the inhibition of acetylcholine (Ach) release at the NMJ by BoNT. Following systemic dissemination, BoNT binds to cholinergic nerve terminals, leading to cranial nerve dysfunction manifested as diplopia, ptosis, dysphonia, and urinary incontinence. Eventually, the symptoms may progress to limb weakness and respiratory failure [18, 19]. The onset of symptoms may vary depending on the amount of toxin ingested, absorbed, and inhaled. BoNT can be absorbed within a few hours or even up to 8 days. The effects of this toxin manifest 12 to 72 hours after its entry into the body, and after 10 days, the severity of the symptoms reaches its peak. Ultimately, the effect of BoNT lasts for about 8 to 12 weeks. Botulism is classified into foodborne botulism, wound botulism, infant botulism, adult intestinal colonization botulism, inhalational botulism, and iatrogenic botulism based on its characteristics and methods of transmission to the body [20, 21]. In fact, botulism is recognized as a rare yet challenging example in the medical world, with the severity and intensity of its symptoms influenced by the type of BoNT serotype, the dose ingested, and the route of absorption. Sufficient knowledge of the different types of botulism and their clinical course not only accelerate the diagnosis and immediate treatment process but can also be a step toward developing more targeted and safer therapies.

Foodborne botulism

It is one of the acute paralytic diseases caused by the consumption of food containing botulinum toxin or spore formation in the intestine and falls under the category of non-communicable diseases. The first manifestations of this disease appear as visual problems such as blurred vision, dry mouth, and dysphagia (difficulty swallowing). Other symptoms may include diarrhea, vomiting, abdominal pain, and nausea. A noteworthy point in this disease is the absence of fever, and fever is only observed in the presence of infectious complications of the disease [15]. According to a study conducted in the United States, approximately 89 percent of foodborne botulism cases were associated with home-canned products, with about 60 percent due to the consumption of contaminated vegetables, 25 percent from the consumption of canned fruits and fish, and the remainder related to other factors [22]. Additionally, the results of research conducted in Iran indicate that traditionally processed fish products, commercially canned products, and finally non-pasteurized dairy products are, respectively, among the most common causes of foodborne botulism outbreaks in Iran. According to studies, the incidence rate of this disease per year among 100,000 native Iranians, broken down by gender, is on average 1.7 cases in men and 3.3 cases in women [23].

Wound botulism

Wound botulism is identified by the observation of clinical symptoms of botulism following trauma, along with the presence of an infected wound and the absence of a history of foodborne illness. Intravenous drug abuse is another factor associated with wound botulism. Since the 1990s in the United States, the use of black tar heroin (BTH) through subcutaneous and sometimes intramuscular injection has led to the spread of this disease, with a reported mortality rate of 13.2% in the country. Soil or wood pulp are among the materials involved in the production and distribution process of BTH, and the sporulation of *C. botuli-*

num also occurs on these materials. As a result, there is a possibility of spores being present on this drug, and subcutaneous injection creates anaerobic conditions for *C. botulinum* and the production of BoNT [24, 25].

Infant botulism and colonization of the adult intestine

Another rare disease caused by the growth and multiplication of *C. botulinum* in the intestinal environment, leading to intestinal immobility, is infant botulism. In the infant's intestine, due to the absence of protective bacterial flora and bile acids that inhibit Clostridium, the bacteria can grow and produce neurotoxin. In most cases, infant botulism occurs in the first few months of life up to one year of age; however, infants are most susceptible between 2 and 4 months of age [26]. If such symptoms occur in children over one year old and adults, it is referred to as adult intestinal colonization botulism. A history of bowel or stomach surgery, certain anatomical bowel abnormalities, Crohn's disease, inflammatory bowel disease, and antimicrobial treatments for foodborne botulism are factors that can lead to intestinal botulism in infants and adults [27].

Inhalational botulism

In some cases, botulinum toxin may act as an inhalational toxin, which requires absorption through the respiratory system. Reports and some studies conducted on animals indicate that inhalation of the toxin leads to illness, and 12 to 72 hours later, symptoms similar to foodborne botulism appear. In this regard, antibiotics have no effect on inhalational and foodborne botulism [28, 29] (*Table 2*).

Table 2 - Comparison of the main types of botulism and examination of the characteristics related to each type.

Main types of botulism	*Cause of the disease*	*Infection source*	*Disease symptoms*	*Disease treatment*	*Prevalence*	*Prevention*
Foodborne botulism	Consumption of foods containing pre-formed toxin (such as canned foods).	Canned foods, homemade products	Common gastrointestinal problems include nausea, vomiting, diarrhea, and neurological symptoms such as muscle weakness, double vision, drooping eyelids, and difficulties in swallowing and speaking.	Treatment with antitoxin and supportive care.	Most prevalent.	Safe canning of foods, boiling canned foods before consumption, avoiding consumption of damaged cans.
Infant botulism	Ingestion of spores and their growth in the intestine.	Soil, dust, and honey.	Constipation (the most common symptom), muscle weakness (floppy baby syndrome), drooping eyelids, general weakness including poor feeding and weak crying.	Treatment with antitoxin.	Common.	Not consuming honey.
Wound botulism	Entry of bacteria into the wound and toxin production.	Infected and contaminated wounds.	Gastrointestinal problems (less common), neurological issues similar to foodborne botulism, wound infection.	Treatment with antitoxin and, in some cases, the use of antibiotics and surgery to remove infected tissue.	Less common than the previous two types.	Rapid wound treatment.

NEUROTOXIC EFFECTS OF BOTULINUM TOXIN

Given that the primary target of BoNT is the inhibition of Ach release from cholinergic nerve terminals, thereby disrupting neural signaling, its main sites of activity and effect include the NMJ, postganglionic parasympathetic neurons, cholinergic sympathetic neurons, and autonomic ganglia, where signal transmission depends on this neurotransmitter [20]. Polysialogangliosides (PSGs) are considered effective receptors in the pathogenesis of *C. botulinum*, such that the mechanism of action of BoNT begins with the binding of the receptor-binding domain HC to the PSGs on the cell surface. Subsequently, the binding of the HC domain of the toxin to other surface receptors, including synaptotagmin (Syt) and glycosylated synaptic vesicle protein 2 (Sv2), occurs with high affinity at the presynaptic levels of cholinergic neurons in a selective and irreversible manner. BoNT types A, D, E, and F bind to Sv2, while BoNT types B and G bind to Syt. So far, no specific protein receptor for serotype C has been identified, and according to observations, BoNT type C binds to liposomes containing phosphoinositides [30, 31]. Subsequently, endocytosis of the toxin-receptor complex occurs within the cell. The release of H^+ ions into the vesicles by proton pumps leads to the acidification of the vesicles and the activation of Ach transporter proteins. This process allows the transfer of cytosolic Ach

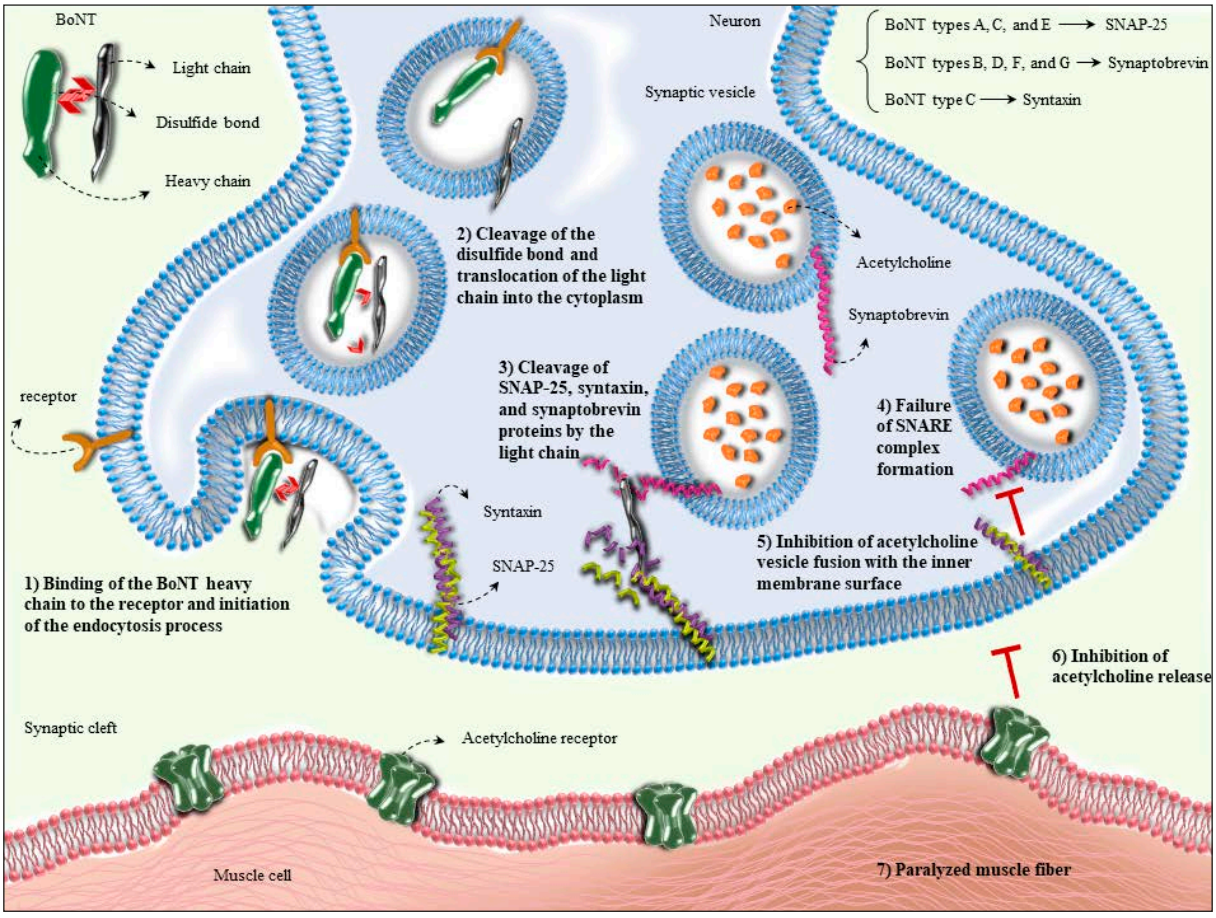

Figure 1. Mechanism of action of botulinum neurotoxin. BoNT: botulinum neurotoxin, SNAP: synaptosomal associated protein, SNARE: soluble N-ethylmaleimide-sensitive factor attachment protein receptor. Figure created by the author using Microsoft PowerPoint, inspired by concepts and illustrations from previously published literature [34, 35].

into the vesicles. BoNT significantly disrupts the subsequent steps in the release of these neurotransmitters. By breaking the disulfide bond between LC and HC, the toxin advances into the cytoplasm [16, 20]. LC remains inactive when bound to other components of the toxin. Subsequently, under the influence of certain cleaving enzymes, including heat shock protein 90 (hsp90) and the thioredoxin reductase–thioredoxin system (TrxR-Trx), LC is released and activated [30]. The joining of LC to the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex occurs after its separation, activation, and entry into the cytoplasm. The release of Ach is facilitated by a complex protein mechanism known as the SNARE complex. In this context, the target proteins vary depending on the type of BoNT serotype. The synaptosomal-associated protein (SNAP) 25 kDa, VAMP, and syntaxin are types of key SNARE proteins. The interaction of LC with these proteins at nerve terminals leads to the inhibition of the binding and fusion of Ach vesicles with the inner surface of the cell membrane [32]. BoNT types F, G, D, and B are responsible for cleaving synaptobrevin or VAMP. SNAP-25 is cleaved by BoNT types A and E, and BoNT type C is also capable of simultaneously cleaving syntaxin and SNAP-25 [33]. The result of this action is the inhibition of SNARE complex formation and consequently the inhibition of neurotransmitter release. Inhibition of Ach release, destruction and chemical denervation of nerves, and ultimately muscle paralysis are accompanied by this [34]. BoNT, due to its large molecular size, is unable to cross the blood-brain barrier and the central nervous system and exerts its effect only on peripheral nerves. Although it may be transmitted to the central nervous system via axons, similar to tetanus toxin (TeNT). However, no direct effects of BoNT on the human central nervous system have been reported so far [18] (*Figure 1*). Overall, the multi-step pathway of BoNT's action, from specific binding and selective targeting to the destruction of the SNARE complex, represents precise control and coordination of cellular processes and a level of molecular finesse that is observable in only a few biological systems. This prominent feature of BoNT, in addition to being the main cause of its paralyzing power, can simultaneously lay the foundation for therapeutic uses in different medical fields.

THE MANAGEMENT AND TREATMENT OF BOTULISM: CURRENT METHODS AND NEW DEVELOPMENTS

Current treatments available for various types of foodborne, wound, intestinal, and inhalational botulism often include supportive measures such as respiratory support, disinfection, and administration of antitoxin. However, the use of antitoxins in treating inhalational botulism has not yet proven to have a positive impact [36]. Other available treatments for botulism include hospitalization, intensive care, and debridement, along with the administration of antibiotics in cases of wound botulism. For instance, intravenous (IV) administration of three million units of penicillin G every four hours, and in case of allergy to it, administration of metronidazole 500 mg every eight hours, are among the antibiotic treatment options. The contraindication of antibiotics in infant botulism is due to the possibility of BoNT dissemination following cell lysis. Parenteral nutrition in cases of severe ileus and bowel irrigation in cases of foodborne botulism without ileus are other treatment options [21]. Guanidine, aminopyridine, and chloroquine are among the drug antagonists that delay the onset of paralysis caused by BoNT by one to two hours, although they do not have a protective effect against its lethal effects. Botulinum antitoxin is considered a specific treatment method for botulism that prevents the production of BoNT, and its administration in the early stages of the disease (24 to 48 hours after the onset of symptoms) prevents the progression of paralysis and respiratory distress. Antibodies or antibody fragments are components of botulinum antitoxin. Among the benefits of using antitoxins in patients are the reduction of recovery time and the decrease in mortality rates associated with it. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) obtained the first botulinum antitoxin serum from the horse First Flight in the 1970s [18, 37, 38]. Heptavalent botulinum antitoxin (HBAT) is an antitoxin active against seven serotypes of BoNT (A, B, C, D, E, F, and G) derived from horse serum, containing 2% immunoglobulin G (IgG) and 90% F(ab')2. It has been approved by the Food and Drug Administration (FDA) for the treatment of symptomatic botulism in adult and pediatric patients. The digestion of antibodies isolated from horse serum into the mentioned fragments by

pepsin leads to a reduction in allergic reactions in patients when administering the antitoxin. Botulism Immune Globulin Intravenous (BIG-IV) is a type of human-derived antitoxin prepared from the plasma of donors vaccinated with the botulinum toxoid vaccine. It is used exclusively for the treatment of botulism in infants under one year of age and neutralizes BoNT types A and B [37, 39-41]. A preclinical study investigated the efficacy of HZ45, a human monoclonal antibody, in the prevention and treatment of BoNT/A poisoning in mice and rabbits. HZ45 actually prevents the entry of the toxin into nerve cells by binding to the HC receptor (HCR), thereby inhibiting the degradation of SNAP-25, which is a key factor in muscle paralysis. The results of this experiment showed that injecting even 0.1 mg of HZ45 into mice before their poisoning with BoNT/A provided 100% protection against 100 LD_{50} of this toxin. Subsequently, the injection of 5 mg of HZ45 into rabbits 4 or 8 hours after their poisoning with 10 LD_{50} of this toxin was able to save all the rabbits, as they did not show any clinical signs related to poisoning. These results indicate the preventive and therapeutic effects of this human monoclonal antibody [42]. Despite recent advances and successes in the treatment of botulism through supportive measures and antitoxins, due to certain therapeutic limitations in different types of botulism and age-related treatment constraints, this disease remains a significant challenge in the fields of medicine, basic research, drug development, and public health. Nevertheless, the high potential of human monoclonal antibodies such as HZ45 in the prevention and treatment of BoNT/A intoxication indicates that the future of botulism therapy is moving toward more specialized drugs with fewer side effects, which could improve standards of care.

INNOVATIVE THERAPEUTIC APPLICATIONS OF BOTULINUM TOXINS

The analgesic effects of BoNTs have been proven due to their various functions, including the inhibition of Ach release at the NMJ site, as well as the suppression of specific neurotransmitters and pain mediators. Blocking the release of Ach by BoNT leads to the disruption of nerve transmission and ultimately results in reduced muscle contractions and pain relief. In addition to the aforementioned effects, BoNT alleviates pain by preventing the peripheral and central release of key pain signaling transmitters, including glutamate, substance P (SP), and calcitonin gene-related peptide (CGRP) [43]. Reduction of inflammation is another therapeutic effect of BoNTs, which is associated with the inhibition of pain mediators' release from peripheral nerve terminals, dorsal root ganglia, and spinal cord neurons [31]. The ability of BoNT to modulate transient receptor potential (TRP) ion channels, sodium and calcium channels, purinergic receptors, neurokinin-1 (NK-1) receptors, and glutamate receptors can lead to pain reduction and relief. Additionally, BoNT interacts with GABAergic pathways (gamma-aminobutyric acid) and the endogenous opioid system, leading to increased inhibitory signaling. These interactions highlight the multifaceted role and therapeutic potential of BoNT in pain management [31]. According to conducted studies, administering a single session of BoNT type A injection according to the Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) protocol in patients with chronic migraine (CM) has significant clinical benefits, including a reduction in the frequency and severity of migraine attacks. Three months after the injection, significant neurophysiological changes are also observable, such that changes in excitability in the trigeminal pain system can be measured and recorded at both the brainstem and cortical levels. This indicates the changes occurring in pain processing pathways, which are due to the positive therapeutic effects of BoNT [44, 45]. Considering the analgesic effects of BoNTs, they can be used as a supportive treatment to significantly reduce pain in cancer patients undergoing surgery and radiotherapy [46]. In another study, the injection of BoNT into epicardial adipose tissue in patients undergoing coronary artery bypass grafting (CABG) surgery resulted in a significant and sustained reduction in the occurrence and frequency of atrial tachyarrhythmia. Based on this, BoNT can be considered a neuromodulator that leads to improved regulation of nerve activity [47]. The use of BoNT type A as a therapeutic option for managing intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) is recommended. Injecting this toxin in such conditions can lead to reduced muscle contractions, improved blood flow, and consequently lower intra-abdominal pressure. Additionally, in more serious conditions where surgical interven-

tions are required, the use of this toxin can help alleviate pain and reduce intra-abdominal pressure before surgery [48]. As previously mentioned, the targeted action, selective paralysis, and reduction of pain signals are the basis of the analgesic and anti-inflammatory therapeutic effects of BoNT. The mentioned therapeutic properties indicate that the intelligent utilization of this toxin can significantly improve the quality of life for patients. These data and evidence clearly confirm the practical value and potential of BoNT in the treatment, management, and clinical care.

IATROGENIC BOTULISM

Iatrogenic botulism is one of the emerging human-made botulisms that can manifest as an unwanted complication after cosmetic or therapeutic procedures. Adverse effects of using botulinum toxin for cosmetic purposes are rare. BoNT type A is one of the commonly used neurotoxins for esthetic and therapeutic purposes. The injection of botulinum toxin is recognized as a first-line treatment for facial muscle spasms (hemifacial spasms) and focal dystonias, such as blepharospasm and cervical dystonia. In 1984, the positive effect of using botulinum toxin in the treatment of blepharospasm was confirmed by Justinus Kerner and his wife, and it was subsequently observed that frown lines had also disappeared. Finally, in 1996, the first case of using this toxin for cosmetic purposes was reported. Strabismus, focal spasticity, overactive bladder, axillary and palmar hyperhidrosis, ptosis, and Frey's syndrome are other conditions for which BoNT can be used for treatment [19, 49].

CONCLUSIONS

C. botulinum, despite its crucial role in causing and initiating botulism, has paradoxically become one of the most valuable therapeutic tools in modern medicine. Examining the mechanism of BoNT's activity in inhibiting Ach release reveals two contrasting yet complementary aspects of this neurotoxin. In other words, BoNT is not only a prime example of the transformation of a "lethal neurotoxin" into a "precise neurological drug", but it is also a symbol of the narrow boundary between toxicity and treatment. Because the discovery of serotype X, with its unique ability to cleave a previously unknown site and reduce toxicity in humans, has put it on track to become an ideal option for developing safer neuromodulating drugs. Traditional approaches to treating botulism using antibodies like HBAT and BIG-IV have been shown to lower death rates; nevertheless, they still have limitations, such as bacterial spore resistance, especially in Group I. Thus, the recently developed HZ45 may eventually take the place of conventional therapies. It is a major treatment advancement because it is a human monoclonal antibody that is 100% effective in neutralizing 100 LD_{50} of type A toxin in animal studies. Further advances in molecular engineering and structural alteration of botulinum toxin may result in the development of a new class of neurotoxins with greater safety and a wider therapeutic window, all the while retaining the capacity to accurately control neurotransmission. With this strategy, the limited neurological and cosmetic uses of botulinum toxin will be extended to new fields such as neurogenic tumors, autonomic disorders, inflammatory diseases, and chronic pain management. Future research should focus on engineering and designing new BONT structures to improve their accuracy and increase their efficiency. Because this genetic engineering and smartening up will likely be able to control their activity so that they are activated only by a specific type of pH, enzyme, or ligand, and remain inactive in the blood under normal conditions. This approach could even transform BONTs into a new type of therapeutic method. Designing BONTs with paradoxical effects and the ability to enhance, inhibit, and repair specific neural pathways could make them an effective therapeutic resource for neurological diseases. If future research focuses on designing or re-engineering BONTs that can be induced, it will be possible to determine the timing and location of this neurotoxin's activity. As a result, precise and safe control of BONT without damaging other pathways can be achieved in the future.

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Author contributions
All Authors contributed in data collection, writing and final draft of the manuscript.

REFERENCES

[1] Keenan S, Niedziela D, Morera-Pujol V, et al. Zoonotic disease classification in wildlife: a theoretical framework for researchers. *Mammal Rev*. 2024; 54(1): 63-77.
[2] Heilmann A, Lacy A, Koyfman A, and Long B. High risk and low prevalence diseases: Botulism. *Am J Emerg Med*. 2024; 82: 174-182.
[3] Erbguth FJ From poison to remedy: the chequered history of botulinum toxin. *J Neural Transm (Vienna)*. 2008; 115(4): 559-565.
[4] Rawson AM, Dempster AW, Humphreys CM, and Minton NP. Pathogenicity and virulence of *Clostridium botulinum. Virulence*. 2023; 14(1): 2205251.
[5] Gutiérrez-Arnal J and Marín C. The Latent Threat in Wild Birds: *Clostridium botulinum. Vet Sci*. 2024; 11: 36.
[6] Liu D. *Clostridium botulinum* and associated neurotoxins, In *Molecular Medical Microbiology (Third Edition)* (Y.-W. Tang, et al., Eds). Academic Press; 2024: 933-944.
[7] Gonzalez-Escalona N and Sharma SK. Closing *Clostridium botulinum* Group I Genomes Using a Combination of Short- and Long-Reads. *Front Microbiol*. 2020; 11: 239.
[8] Wang S, Zhang H, Xue Y, Yang Y, and Yuan L. Research Progress on the Detection Methods of Botulinum Neurotoxin. *Toxins*. 2025; 17(9): 453.
[9] Harris RA and Dabritz HA. Infant Botulism: In Search of *Clostridium botulinum* Spores. *Curr Microbiol*. 2024; 81(10): 306.
[10] Thirunavukkarasu N, Johnson E, Pillai S, et al. Botulinum Neurotoxin Detection Methods for Public Health Response and Surveillance. *Front Bioeng Biotechnol*. 2018; 6: 80.
[11] Tiwari A and Nagalli S. *Clostridium botulinum* Infection(Archived), In *StatPearls*, StatPearls Publishing: Treasure Island (FL); 2024.
[12] Gregg Brieana M, Matsumura T, Wentz Travis G, et al. Botulinum neurotoxin X lacks potency in mice and in human neurons. *mBio*. 2024; 15(3): e03106-03123.
[13] Masuyer G, Zhang S, Barkho S, et al. Structural characterisation of the catalytic domain of botulinum neurotoxin X high activity and unique substrate specificity. *Sci Rep*. 2018; 8(1): 4518.
[14] Chimienti S, Di Spirito M, Molinari F, et al. Botulinum Neurotoxins as Two-Faced Janus Proteins. *Biomedicines*. 2025; 13(2): 411.
[15] Chaidoutis E, Keramydas D, Papalexis P, et al. Foodborne botulism: A brief review of cases transmitted by cheese products (Review). *Biomed Rep*. 2022; 16(5): 41.
[16] Grenda T, Grenda A, Krawczyk P, Kwiatek K. Botulinum toxin in cancer therapy - current perspectives and limitations. *Appl Microbiol Biotechnol*. 2022; 106(2): 485-495.
[17] Corsalini, M., F. Inchingolo, G. Dipalma, et al. Botulinum Neurotoxins (BoNTs) and Their Biological, Pharmacological, and Toxicological Issues: A Scoping Review. *Applied Sciences*. 2021; 11(19): 8849.
[18] Rao AK, Sobel J, Chatham-Stephens K, Luquez C. Clinical Guidelines for Diagnosis and Treatment of Botulism, 2021. *MMWR Recomm Rep*. 2021; 70(2): 1-30.
[19] Islam MR, Akash S, Emran TB, Rauf A. The recent outbreak of Iatrogenic botulism: point of view from the present world: editorial. *Ann Med Surg*. 2023; 85(6): 2289-2290.
[20] Nigam PK, Nigam A. Botulinum toxin. *Indian J Dermatol*. 2010; 55(1): 8-14.
[21] Jeffery IA, Nguyen AD, S. Karim S. Botulism, In *StatPearls*, StatPearls Publishing: Treasure Island (FL); 2025.
[22] Gangarosa EJ, Donadio JA, Armstrong RW, et al. Botulism in the United States, 1899-1969. *Am J Epidemiol*. 1971; 93(2): 93-101.
[23] Khorasan MRM, Rahbar M, Bialvaei AZ, et al. Prevalence, Risk Factors, and Epidemiology of Foodborne Botulism in Iran. *J Epidemiol Glob Health*. 2020; 10(4): 288-292.
[24] Neeki MM, Dong F, Emond C, et al. Early diagnosis and critical management of wound botulism in the emergency department: a single center experience and literature review. *Int J Emerg Med*. 2021; 14(1): 56.
[25] Burningham MD, Walter FG, Mechem C, Haber J, Ekins BR. Wound botulism. *Ann Emerg Med*. 1994; 24(6): 1184-1187.
[26] Dilena R, Pozzato M, Baselli L, et al. Infant Botulism: Checklist for Timely Clinical Diagnosis and New Possible Risk Factors Originated from a Case Report and Literature Review. *Toxins*. 2021; 13(12): 860.
[27] Harris RA, Anniballi F, and Austin JW. Adult Intestinal Toxemia Botulism. *Toxins (Basel)*. 2020; 12(2): 81.
[28] Davis LE. Botulism. *Curr Treat Options Neurol*. 2003; 5(1): 23-31.
[29] Park, J.B. and L.L. Simpson. Inhalational poisoning by botulinum toxin and inhalation vaccination with its heavy-chain component. *Infect Immun*. 2003; 71(3): 1147-1154.
[30] Choudhury S, Baker MR, Chatterjee S, and Kumar H. Botulinum Toxin: An Update on Pharmacology and Newer Products in Development. *Toxins (Basel)*. 2021; 13(1): 58.
[31] Hosseindoost S, Askari Rad M, Inanloo SH, et al. The analgesic effects of botulinum neurotoxin by mod-

ulating pain-related receptors; A literature review. *Mol Pain*. 2024; 20: 17448069241275099.
[32] Dressler D, Saberi FA, Barbosa ER. Botulinum toxin: mechanisms of action. *Arq Neuropsiquiatr*. 2005; 63(1): 180-185.
[33] Arnon S, Schechter R, Inglesby T, et al. Botulinum Toxin as a Biological WeaponMedical and Public Health Management. *JAMA*. 2001; 285(8): 1059-1070.
[34] Gazerani P. How Does Botulinum Toxin Inhibit Itch? *Toxins*. 2022; 14(10): 701.
[35] Chimienti, S., M. Di Spirito, F. Molinari, et al. Botulinum Neurotoxins as Two-Faced Janus Proteins. *Biomedicines*. 2025; 13(2): 411.
[36] Robinson, R.F. and M.C. Nahata. Management of botulism. *Ann Pharmacother* 2003; 37(1): 127-131.
[37] Ni SA, Brady MF. Botulism Antitoxin, In *StatPearls*, StatPearls Publishing: Treasure Island (FL); 2025.
[38] Mayers C, Holley J, and Brooks T. Antitoxin therapy for botulinum intoxication. *Rev Med Microbiol*. 2001; 12: 29-37.
[39] Pelletier, J.P.R. and F. Mukhtar. Passive Monoclonal and Polyclonal Antibody Therapies, In *Immunologic Concepts in Transfusion Medicine* (R.W. Maitta, Editor, Elsevier; 2020; 251-348.
[40] (CDC), C.f.D.C.a.P. Investigational heptavalent botulinum antitoxin (HBAT) to replace licensed botulinum antitoxin AB and investigational botulinum antitoxin E. *MMWR*. 2010; 59(10): 299.
[41] Parrera GS, Astacio H, Tunga P, et al. Use of Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-(Equine) (BAT(®)) in Clinical Study Subjects and Patients: A 15-Year Systematic Safety Review. *Toxins (Basel)*. 2021; 14(1): 19.
[42] Yu CH, Song YJ, Song DH, et al. An Effective Prophylactic and Therapeutic Protection Against Botulinum Type A Intoxication in Mice and Rabbits Using a Humanized Monoclonal Antibody. *Toxins*. 2025; 17(3): 138.
[43] Mittal, SO, Jabbari B. Botulinum Neurotoxins and Cancer - A Review of the Literature. *Toxins (Basel)* 2020; 12: 32.
[44] Sebastianelli, G., F. Casillo, A. Di Renzo, et al. Effects of Botulinum Toxin Type A on the Nociceptive and Lemniscal Somatosensory Systems in Chronic Migraine: An Electrophysiological Study. *Toxins (Basel)* 2023; 15(1): 76.
[45] Shaterian N, Shaterian N, Ghanaatpisheh A, et al. Botox (OnabotulinumtoxinA) for Treatment of Migraine Symptoms: A Systematic Review. *Pain Res Manag*. 2022; 2022: 3284446.
[46] Lippi L, de Sire A, Turco A, et al. Botulinum Toxin for Pain Relief in Cancer Patients: A Systematic Review of Randomized Controlled Trials. *Toxins (Basel)*. 2024; 16(3): 153.
[47] Romanov A, Pokushalov E, Ponomarev D, et al. Long-term suppression of atrial fibrillation by botulinum toxin injection into epicardial fat pads in patients undergoing cardiac surgery: Three-year follow-up of a randomized study. *Heart Rhythm*. 2019; 16(2): 172-177.
[48] Adeboye T, Kulkarni G, Atkinson T, Hammond T. 913 Role of Botulinum Toxin in the Medical Management of Respiratory Distress Secondary to Suspected Intraabdominal Hypertension After Abdominal Wall Reconstruction. *Br J Surg*. 2023; 110(Supplement_7): znad258.294.
[49] Fung HT, Chan KM, Lam SKT. A review on iatrogenic botulism. *Hong Kong J Emerg Med*. 2020; 27(6): 356-367.