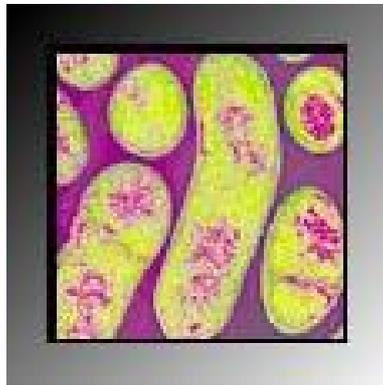


CLOSTRIDIUM BOTULINUM



**International Programme on Chemical Safety
Poisons Information Monograph 858
Bacteria**



WORLD HEALTH ORGANIZATION

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1. NAME

1.1 Scientific name

Clostridium botulinum

1.2 Family

Clostridium
Endospore-forming gram-positive bacilli

1.3 Common names and synonyms

- Botulinum toxin;
- Toxinum botulinum;
- Botulinum A toxin haemagglutinin complex;
- Oculinum® (Allergan Pharmaceuticals, USA);
- Botox® (produced by Allergan Pharmaceuticals, USA);
- Dysport® (produced by Ipsen, UK);
- Jad kielbasiany (Polish);

2. SUMMARY

2.1 Main risks and target organs

Botulism is characterised by symmetrical, descending, flaccid paralysis of motor and autonomic nerves usually beginning with cranial nerves. It occurs when neuromuscular transmission is interrupted by a protein neurotoxin produced by the spore-forming, obligate anaerobic bacterium *Clostridium botulinum*. Paralysis begins with the cranial nerves, then affects the upper extremities, the respiratory muscles, and, finally, the lower extremities in a proximal-to-distal pattern. In severe cases, extensive respiratory muscle paralysis leads to ventilatory failure and death unless supportive care is provided.

2.2 Summary of clinical effects

There are five clinical categories of botulism: 1) foodborne botulism; 2) wound botulism; 3) infant botulism; 4) adult infectious botulism; 5) inadvertent, following botulinum toxin injection.

Foodborne botulism

Onset generally occurs 18 to 36 hours after exposure (range, 6 hours to 8 days). Initial symptoms can include nausea, vomiting, abdominal cramps or diarrhoea. After the onset of neurologic symptoms, constipation is typical. Dry mouth, blurred vision, and diplopia are usually the earliest neurologic symptoms. They are followed by dysphonia, dysarthria, dysphagia, and peripheral muscle weakness. Symmetric descending paralysis is characteristic of botulism.

Wound botulism

This can be defined as clinical evidence of botulism following lesions, with a resultant infected wound and no history suggestive of foodborne illness. Except for the gastrointestinal symptoms, the clinical manifestations are similar to those seen in foodborne botulism. However, the incubation period is much longer as time is required for the incubation of spores, growth of clostridium and release of toxins (4 to 14 days).

Infant botulism

This is caused by the absorption of toxin produced by *Clostridium botulinum* that colonize the intestinal tracts of infants under one year of age. It is often associated with ingestion of honey and the first clinical sign is usually constipation. After a few weeks, progressive weakness and poor feeding are observed. The weakness is symmetrical and descending. It evolves over hours or several days. The infant is afebrile and has a weak cry, has either absent or diminished spontaneous movements, decreased sucking, floppy head and decreased motor response to stimuli. The autonomic nervous system manifestations include dry mucous membranes, urinary retention, diminished gastro-intestinal motility, fluctuation of heart rate, and changes in skin colour. Duration of hospitalisation may last from a few days to six months.

Adult infectious botulism

It occurs as a result of intestinal colonization with *C. botulinum* and in vivo toxin production in a manner similar to that of infant botulism. These patients often have a history of abdominal surgery, achlorhydria, Crohn's disease or recent antibiotic treatment. The disease may simulate a Guillain-Barré Syndrome.

Inadvertent botulism

This has been reported in patients who have been treated with intramuscular injections of botulinum toxin. Marked clinical weakness is observed as well as electrophysiologic abnormalities.

2.3 Diagnosis

Foodborne botulism

This should be suspected in a patient with acute onset of gastro-intestinal symptoms associated with autonomic (dry mouth, difficulty focusing eyes) and cranial nerves dysfunction (ptosis, diplopia, dysarthria, dysphagia). A history of home-prepared or home-preserved food (often, inadequately pasteurized vegetables) and similar symptoms in people who have shared the same food increases likelihood of the diagnosis. The initial diagnosis should be made on the basis of history and physical findings. Confirmatory tests may take days to be performed. Serum, stools and suspected food should be tested for the presence of botulism. The mouse inoculation test is still the most reliable method. Stool specimens should be cultured for *C. botulinum* as a confirmatory test. Isolation of *C. botulinum* organism devoid of toxin from the suspected food has little significance.

Wound botulism

Specimens of wound exudate, a tissue sample, or a swab sample should be obtained for anaerobic culture in addition to a serum toxin assay. A stool specimen should be obtained in order to exclude food or intestinal colonization as sources of toxin.

Infant botulism

This should be suspected in an infant with constipation, poor feeding, diminished sucking and crying ability, neck and peripheral muscle weakness, or ventilatory distress. Stool cultures for *C. botulinum* and testing for the presence of toxin in the stool should be performed in such patients.

Adult infectious botulism

This is a rare disease and should be suspected in patients with some abnormality of the gastro-intestinal tract who develop cranial nerve autonomic dysfunction, and muscular weakness. Stool cultures for *C. botulinum* and testing for the presence of toxin should be performed. Endogenous antibody production to botulinum toxin has been described.

Inadvertent botulism

This may be suspected in patients with recent history of botulin A toxin injection, especially into big muscles for systemic effect, or perhaps, in a suicide attempt.

2.4 First Aid Measures and Management Principles

Foodborne botulism

Emptying the stomach by gastric lavage or induction of vomiting with syrup of ipecac *could* be considered if the suspected food ingestion was recent (within 1 hour). It should not be attempted if neurological symptoms are already present.

Administer activated charcoal and a cathartic (such as sorbitol) but not magnesium salts since magnesium may potentiate neuromuscular block. Maintain airway and assist ventilation if required.

Obtain arterial blood gases. Monitor respiration closely since respiratory arrest can occur abruptly.

Administer Trivalent ABE antitoxin (7500 IU of type A, 5500 IU of type B, and 8500 IU of type E antitoxins) per patient. First test for serum sensitivity by injecting 0.1 mL of a 1:10 dilution of antitoxin in saline intradermally. Monitor for any reaction for 15 minutes before administering a full dose. If a reaction occurs the dose and rate of infusion must be reduced and the reaction must be treated. A single dose of antitoxin is usually sufficient.

Wound botulism

Because of the slow recovery period, trivalent antitoxin administration may need to be repeated.

Infant botulism

Equine botulinum antitoxin is not used in infant botulism because of the potential risk of anaphylaxis, serum sickness, or the sensitization of the infant to horse antigen. A human-derived antitoxin product (immune globulin) is being evaluated in a controlled trial in California (USA) for use in infants. For information on the Infant Botulism Prevention Programme contact the California Department of Health Services at (510) 540-2646 (24 hours).

Adult infectious botulism

Trivalent antitoxin may need to be readministered after the first dose because of the prolonged evolution.

2.5 Poisonous parts

Botulism is caused by a group of anaerobic spore-forming organisms called *Clostridium botulinum*. This is classified as a single species but consists of at least three genetically distinguishable groups of organisms that have been recognized as toxic for humans. They share the ability to produce neurotoxins with similar pharmacological activities but diverse serologic properties. The toxin types are classified as A, B, C, D, E, F and G. Human botulism has been described with the strains of *Clostridium botulinum* that produce toxin types A, B and E. Less frequently, cases involving type F toxin produced by *C. baratii* and type E toxin produced by *C. butyricum* have been published

2.6 Main Toxins

Although the seven neurotoxins (A, B, C, D, E, F and G) are genetically distinct, they possess similar molecular weights and have a common subunit structure. The complete amino acid sequences of the various serotypes are becoming known. Regions of sequence homology among the serotypes and between botulinum toxins and tetanus toxin, suggest that they all employ similar mechanisms of action.

The toxins are synthesized as single chain polypeptides with a molecular mass of approximately 150 kDa. In this form, the toxin molecules have relatively little potency as neuromuscular agents. Neurotoxin activation requires a two-step modification in the tertiary structure of the protein.

3. CHARACTERISTICS

3.1 Description of the bacterium

3.1.1 Special identification features

Clostridium botulinum is a gram positive, obligate anaerobic, spore-forming, rod-shaped bacterium.

3.1.2 Habitat

Clostridium botulinum organisms are commonly found in soils and marine sediments throughout the world.

3.1.3 Distribution

C. botulinum may be found in any region of the world. Since it is found in the soil, it may contaminate vegetables cultivated in or on the soil. It also colonizes the gastro-intestinal tract of fishes, birds and mammals.

3.2 Poisonous parts

All clostridial neurotoxins are synthesized as a single inactive polypeptide chain of 150 kDa without a leader sequence and hence are presumably released from the cell by bacterial lysis (Schiavo et al, 1995).

The organisms that can produce botulinum neurotoxin are diverse. Even though they were shown to have different phenotypic characteristics, all organisms capable of producing botulinum neurotoxin become classified as *Clostridium botulinum* (Prevot, 1953).

These are the characteristic of clostridia capable of producing botulinum neurotoxin:

C. BOTULINUM GROUP						
	I	II	III	IV*	<i>C. baritii</i>	<i>C. butyricum</i>
Toxin type	A, B, F	B, E, F	C, D	G	F	E
Growth temperature (°C)						
Optimum	35–40	18–25	40	37	30–37	30–45
Minimum	12	3.3	15			10

* *C. argentinense* has been proposed for this group (Hatheway, 1995).

3.3 The Toxin

3.3.1 Name(s)

Human botulism is primarily caused by *Clostridium botulinum* that produce toxin type A, B and E. Type F toxin produced by *Clostridium baritii* and type E toxin produced by *Clostridium butyricum* have also been implicated in human botulism.

Strains of *C. botulinum* that produce type C or type D toxin for the most part cause botulism only in non-human species (Shapiro et al, 1998).

3.3.2 Description, chemical structure, stability

All clostridial neurotoxins are synthesized as a single inactive polypeptide chain of 150 kDa without a leader sequence and hence are presumably released from the cell by bacterial lysis. Bacterial or tissue protease cleaves these toxins within an exposed highly protease-sensitive loop and generates the active di-chain neurotoxins composed of a heavy chain (H, 100 kDa) and a light chain (L, 50 kDa) joined by disulphide bonds, that is associated with one atom of zinc. This interchain S-S bond plays a critical role in cell penetration, and its cleavage by reduction abolishes toxicity (Schiavo et al, 1995).

The heavy chain can be divided functionally into an amino terminal domain (Hn) and a carboxyl terminal domain (Hc) (Halpern & Neale, 1995).

The light chain (amino acids 1-448) acts as a zinc endopeptidase, with proteolytic activity concentrated at the N-terminal end. The heavy chain (amino acids 449-1280) provides cholinergic specificity and promotes light chain translocation across the endosomal membrane of the neurotransmitter.

If the disulphide bond that links the two chains is broken before the toxin is internalised in the cell, the light chain cannot enter the axon terminal membrane, and there is a virtually complete loss of toxicity (Brin, 1997) (see 7,1 Mode of action).

3.3.3 Other physicochemical characteristics

The toxin in the complex is rather stable, especially under acidic conditions (pH 3,5 to 6,5), but the complex dissociates under slightly alkaline conditions and the biological activity is readily inactivated in this state. The neurotoxin can be separated from the non-toxic components and purified by ion-exchange chromatography (Midura, 1996). Although botulinum spores are relatively heat resistant the toxin itself is heat sensitive. Heating it at 80°C for 30 minutes or 100°C for 10 minutes destroys the active toxin (Slovic & Jones, 1998).

3.4 Other Chemical Contents of the bacteria

C. botulinum spores produced by all strains are highly heat resistant. Toxins produced by some *Clostridium botulinum* bacteria are non-proteolytic, which means that affected food may look and smell normal (Cherington, 1998).

4 USES/CIRCUMSTANCES OF POISONING

4.1 Uses

4.1.1 Uses

Neurotoxin: Pharmaceutical for human use (agent acting on the nervous system); Other Bacterium: Warfare/Anti riot agent: biological warfare agent

4.1.2 Description

Botulin toxin A is used in the treatment of spastic muscular conditions such as torticollis, cervical and upper limb dystonia, childhood strabismus, apraxia of eye-lid opening, hemifacial spasm, writer's cramp, spasticity in cerebral palsy in children, but also in the treatment of hyperhidrosis (Munchau & Bhatia, 2000). It is also used for cosmetic purposes to reduce wrinkles.

4.2 High risk circumstances

There are 5 clinical categories of botulism of which the foodborne type is the most common. Canned or bottled food, particularly homemade, may contain botulinum (Cherington, 1998; Slovic & Jones, 1998).

Foodborne botulism

Home-prepared and home-preserved foods (often inadequately pasteurized vegetables, despite the name coming from the Latin *botulus* = *sausage*) are the most frequent cause of poisoning. The particular foods involved vary according to geographical and cultural peculiarities:

- Strong-smelling preserved bean curd in China (Ying & Shuyan, 1986)
- Canned vegetables in U.S.A (MacDonald et al, 1986)

- Meat from marine animals or fish/fish eggs fermented in traditional ways in Canada (Hauschild & Gauvreau, 1985)
- Preserved ham (Lecour et al, 1988 ; Roblot et al, 1994)
- Home-made sauces; baked potatoes sealed in aluminum foil; cheese sauce; sautéed onions held under a layer of butter; garlic in oil; traditionally prepared salted or fermented fish

The vehicles for botulism change with time even in the same country. For example, in USA, new sources have been described in recent years (Shapiro et al, 1998; Townes et al, 1996). This influences the type of toxin involved (Hatheway, 1995; Hauschild, 1992). Cases recorded in 38 countries between 1951 and 1989 show that 72 % of the outbreaks and 48 % of the cases were reported from Poland. Of the 2622 outbreaks in which the toxin type was determined, 34 % were type A, 52 % type B, and 12 % type E. Two incidents of type F foodborne botulism were reported during this period.

Wound botulism

A review of 40 cases of wound botulism published in the literature (Mechem & Walter, 1994) showed that most of these cases involved puncture wounds, open fractures, lacerations, crush injuries, shotgun wounds, drug abuse (abscesses), and surgical incisions. In some cases, no site of inoculation could be found. In the 13 cases where the toxin was isolated, 11 had type A, one had type B and the type of the toxin was not mentioned in one case. The use of Mexican black tar heroin was responsible for a cluster of cases in California (Anderson et al, 1997; Maselli et al, 1997).

Infant botulism

In most cases, the source of ingestion is unknown but in 15 % of cases, ingestion of honey is suspected (Shapiro et al, 1998).

The toxin type in infant botulism is generally either A or B, and the organisms are group I *C. botulinum*. Two cases have been reported in USA involving strain *C. baratii* that produce a neurotoxin similar to type F and two cases have been reported in Italy caused by strains *C. butyricum* that produce type E neurotoxin (Hatheway, 1995).

Adult infectious botulism

Two patients with clinical signs and symptoms of botulism yielded *C. botulinum* type A in their stool cultures for as long as 119 and 130 days after onset of illness (Hatheway, 1995).

Factors associated with this form of botulism were bowel surgery, Crohn's disease, or previous contaminated food exposure without illness.

Inadvertent botulism

This is a more recent form of botulism caused by the use of the toxin to treat dystonic and other movement disorders (Cherington, 1998; Bhatia et al 1999). In patients with torticollis treated with botulinum A toxin injected into the neck muscles dysphagia may develop from toxin penetrating the nearby pharyngeal muscles. The penetration of the toxin to distant muscles or generalized weakness due to systemic distribution of the toxin is rare (Bakheit et al, 1997; Bhatia et al, 1999).

4.3 High risk geographical areas

Out of 449 outbreaks with 930 cases reported in literature reviews (Hatheway, 1995), 72 % of the outbreaks and 48 % of the cases occurred in Poland.

Clostridial spores are resistant to heat and may survive the home-preserving process at temperatures below 120 °C. At high altitude boiling food prior to canning may not provide a high enough temperature to destroy the spores (Cherington, 1998).

Traditional food preparation and preservation is a major factor in the production of foodborne botulism (Hauschild, 1992). Non-acidic foods need to be pasteurized twice, at 24h intervals, to kill the bacteria generated from the surviving spores (Cherington, 1998).

5. ROUTES OF EXPOSURE

5.1 Oral

Foodborne botulism

This is caused by ingestion of food contaminated by a preformed neurotoxin of the bacterium *Clostridium botulinum*. Home-preserved foods containing fish, vegetables, or potatoes are often involved in outbreaks of botulism. High acid content foods are rarely involved. *C. botulinum* spores are heat-resistant but the toxin is heat-labile. Boiling food to ensure thorough heating of the interior should destroy the toxin (Cherington, 1998).

Infant botulism

This is a result of colonization of the intestinal tract after ingestion of spores of *C. botulinum*. The infant intestinal tract often lacks both the protective bacterial flora and the clostridium-inhibiting bile acids found in normal adult intestinal tract. Honey was found to be the vehicle of the spores in 26 cases (Arnon, 1992). Most cases occur before the age of 6 months. Microbiologic surveys of honey products have reported the presence of clostridial spores in up to 25 % of products. For this reason, honey should not be given to children during the first year of life (Cherington, 1998; Hatheway, 1995; Shapiro et al, 1998).

Adult infectious botulism

In most cases, the responsible food could not be identified. One adult appeared to develop botulism 47 days after exposure to a food that caused botulism in four other family members (Hatheway, 1995).

5.2 Inhalation

Studies in monkeys indicate that, if aerosolised, botulinum toxin also can be absorbed through the lung (Shapiro et al, 1998).

Three cases of botulism in laboratory workers have been ascribed to inhalation of the toxin (Cherington, 1998).

Recent concern about the use of *C. botulinum* neurotoxin aerosol in a terrorist attack has drawn attention to the potential risk to public health and the need for preventive measures to be developed (Steffen et al, 1997). This prompted the development of a heptavalent (type A-G) equine botulinum immune globulin (BIG) containing purified F(ab)₂ by the United States army (Middlebrook & Brown, 1995).

5.3 Dermal

Neither the spores nor the neurotoxins are able to penetrate intact skin. However damaged skin may be affected (Slovic & Jones 1998)

5.4 Eye

No data available.

5.5 Parenteral

Wound botulism

The first case of wound botulism was published in 1951. The case occurred in 1943 and involved an adolescent girl who had sustained an open fracture of her left leg and right ankle following a fall from a building (Mechem & Walter, 1994). At the time of that review, a total of 40 cases had been reported in the English-language literature.

Inadvertent botulism

Several cases have been reported following intramuscular administration of the toxin for therapeutic purposes (Cherington, 1998; Bhatia et al 1999).

5.6 Others

No information available.

6. KINETICS

6.1 Absorption by route of exposure

No data available

6.2 Distribution by routes of exposure

Botulinum toxins are absorbed from the intestinal tract or the infected wound site and are carried via the lymphatic system, and from the intestinal tract by the bloodstream to the neuromuscular endings. Toxin types differ in their affinity for nerve tissue, with type A having the greatest affinity (Midura, 1996). The toxin must enter the nerve ending to exert its effect. Binding of toxin to both peripheral and central nerves is selective and saturable. Pharmacologic and morphologic data suggest that internalisation is via a receptor-mediated endocytotic/lysosomal vesicle pathway. The process is independent of Ca^{++} concentration, is partially dependent on nerve stimulation, and is energy dependent (Brin, 1997).

6.3 Biological half-life by routes of exposure

No data available.

6.4 Metabolism

No data available.

6.5 Elimination and excretion

No data available.

7. TOXINOLOGY

7.1 Mode of action

Botulinum neurotoxin reaches nerve terminals at the neuromuscular junction, where it binds to the neuronal membrane, moves into the cytoplasm of the axon terminal, and acts to block excitatory synaptic transmission, leading to flaccid paralysis (Halpern & Neale, 1995). There are three steps involved in toxin mediated paralysis: 1) internalisation 2) disulphide reduction and translocation 3) inhibition of the neurotransmitter release (Brin, 1997). The toxin must enter the nerve ending to exert its effect. Binding of toxin to both peripheral and central nerves is selective and saturable. The C-terminal half of the heavy chain determines cholinergic specificity and is responsible for binding, while the light chain is the intracellular toxic moiety. If the disulphide bond that links the two chains is broken before the toxin is internalised by the cell, the light chain cannot enter and there is virtually complete loss of toxicity (Brin, 1997).

The toxin blocks the release of acetylcholine but not its synthesis or storage. Botulinum toxin is a zinc endopeptidase specific for protein components of the neuroexocytosis apparatus. It cleaves synaptobrevin, a membrane protein of synaptic vesicles. The types A, C and E act on proteins of the presynaptic membrane. Types A and E cleave SNAP-25 while serotype C cleaves syntaxin (Schiavo et al, 1995; Montecucco et al, 1996).

7.2 Toxicity

7.2.1 Human data

7.2.1.1 Adults

Comprehensive reviews of the epidemiology, clinical features and management principles have been published in recent years (Hauschild, 1992; Hatheway, 1995; Cherington, 1998; Shapiro et al, 1998; CDC, 1998).

Foodborne botulism

New food items were involved in outbreaks like home-made sauce, baked potatoes sealed in aluminium foil, cheese sauce, sautéed onions held under a layer of butter, garlic in oil, and traditionally prepared salted or fermented fish. The use of modern plastic containers introduced a new risk factor in the ingestion of traditional food in the arctic regions (Hauschild, 1992; Proulx et al, 1997).

A recent comparison of the severity of botulism by toxin type found that endotracheal intubation was required for 67 % of type A patients, 52 % of type B, and 39 % of type E (Woodruff et al, 1992). Severity scores for classification of botulism have been proposed (Roblot et al, 1994).

Wound botulism

This has also been reviewed from the published literature and from a cluster of cases related to the use of black tar heroin (Burningham et al, 1994; Crawford, 1994; Maselli et al, 1997; Anderson et al, 1997).

Adult infectious botulism

This has also been described with greater frequency in recent years (Hatheway, 1995; Shapiro et al, 1998; Cherington, 1998). It is generally associated with abdominal surgery, gastro-intestinal diseases or asymptomatic exposure to contaminated food. It may be hypothesised that the use of anti H₂ histamine medication in these patients may favour the intestinal colonization by *C. botulinum*

since the toxin complex is stable under acidic conditions but dissociates under slightly alkaline conditions.

7.2.1.2 Children

Infant botulism has also been the subject of comprehensive review (Midura, 1996; Glatman-Freedman, 1996; Pickett et al, 1976; Long, 1984; Arnon, 1992; Wiggington & Thill, 1983). The ingestion of honey has been implicated in many cases but the source of contamination is frequently unknown. It occurs among children less than one year of age and mostly in the first six months of life. It has a wide spectrum of severity. Some infants manifest with only mild symptoms and may go unrecognised while other cases present as sudden infant death syndrome. Hospitalisation averages approximately five weeks, but may last up to six months. Infant botulism has been reported from countries all over the world except Africa. Most cases were reported in the United States. The toxin types involved in these cases were A and B in approximately the same proportion.

7.2.2 Relevant animal data

The parenteral median LD₅₀ of botulinum toxin in monkeys and mice is 0.4ng/kg (Gill 1982).

Botulism also occurs in animals. The clinical features are essentially the same as in humans. The toxin involved in these cases was either C or D (Hatheway, 1995). A detailed review of the subject has been published (Smith & Sugiyama, 1988).

Animal models were used to evaluate the efficacy of the antitoxins (Middlebrook & Brown, 1995). Guinea pigs were given 20 IU human botulinum immune globulin per kilogram either 4 hours before or 4 to 8 hours after an oral challenge of type A toxin, and all survived with no clinical signs.

Guinea pigs treated with 1 IU/kg trivalent botulism antitoxin were completely protected from subcutaneous toxin challenge, although protection decreased when antibody was given post challenge.

Supportive care improves the efficacy of botulinum antibody therapy in monkeys.

Infant botulism

Using a mouse model system of intestinal colonization it was demonstrated that the intestinal microflora of adult animals ordinarily prevents colonisation of the intestines by *C. botulinum* (Moberg & Sugiyama, 1979). The infective dose of spores for infant mice was much smaller than that of their antibiotic-treated adult counterparts; the 50 % infective dose for normal infant mice was only 700 spores (Midura, 1996). In one experiment 10 spores were sufficient to infect an infant mouse (Sugiyama & Mills, 1978).

7.2.3 Relevant in vitro data

Detailed reviews of the chemistry, pharmacology, toxicity, immunology and mechanism of action of botulinum neurotoxins have been published in recent years (Halpern & Neale, 1995; Middlebrook & Brown, 1995; Schiavo et al, 1995; Montecucco et al, 1996; Brin, 1997; Coffield et al, 1997). The nucleotide sequence for all seven toxin types has been elucidated (Shapiro et al, 1998).

7.3 Carcinogenicity

No data available.

7.4 Teratogenicity

There is no evidence to date that the fetus is at risk of neonatal botulism when the mother is affected by botulism (Cherington, 1998). There are a few case reports in the literature where the mother acquired botulism during pregnancy. In no case there was there evidence of transport of the toxin across the placental barrier.

7.5 Mutagenicity

No data available.

7.6 Interactions

Aminoglycoside antibiotics potentiate the neuromuscular blockade induced by botulinum toxins both in the human experience of infant botulism and the mouse model (Wang, 1984). Cathartic agents containing magnesium should be avoided because of the theoretical concern that increased magnesium levels may enhance the action of botulinum toxin (Shapiro et al, 1998).

8. TOXICOLOGICAL ANALYSES AND BIOMEDICAL INVESTIGATIONS

8.1 Material sampling plan

8.1.1 Sampling and specimen collection

Specimens of serum, faeces, vomitus and gastric contents, together with implicated foods should be collected for testing for toxin and the presence of *C. botulinum* (Shapiro et al, 1998).

In wound botulism wound exudate, debrided tissue, or a swab sample should be obtained for anaerobic culture. Serum should also be collected for serum toxin assay and a stool specimen should be collected to exclude foodborne botulism. In infant botulism, stools should be collected for culture and toxin identification.

Serum should be collected before antitoxin is given, otherwise there may be a false negative result. If possible at least 3ml of serum should be collected, although as little as 0.5ml may be sufficient. A larger volume, ideally 10-15ml, will allow specific identification of the botulinum toxin involved and repeat testing if necessary (CDC, 1998)

8.1.1.1 Toxicological analyses

The mouse inoculation test is still the most reliable method. The type of toxin, particularly A, B, and E can be detected by injecting the specific antitoxin in combination with the patient's serum into the mouse (Griffin et al, 1997).

8.1.1.2 Biomedical analyses

The differential diagnosis of botulism with other neurological diseases may require rapid repetitive electromyography, lumbar puncture, edrophonium chloride testing,

magnetic resonance imaging or computed tomography of the brain (Shapiro et al, 1998; Cherington, 1998).

8.1.2 Storage of laboratory samples and specimens

8.1.2.1 Toxicological analyses

All specimens except those from wounds should be refrigerated, preferably not frozen, and examined as soon as possible. Wound specimens should be placed in anaerobic transport devices and sent to the laboratory without refrigeration (CDC, 1998).

Food should be left in its original container if possible or placed in a labelled, unbreakable, sterile container.

8.1.3 Transport of laboratory samples and specimens

8.1.3.1 Toxicological analyses

Samples should be conveyed to the laboratory as quickly as possible. The samples should be packed in sterile, leakproof containers. If they have to be sent a long distance then the samples should be placed in insulated shipping containers with refrigerant. If a delay of several days is likely then serum and stool samples should be frozen and packed in dry ice. Packaging should be adequately labelled to indicate that the contents are a biological hazard (CDC, 1998).

8.2 Biomedical investigations and their interpretation

8.2.1 Biochemical analysis

8.2.1.1 Other fluids

CSF is normally clear, although a slightly elevated protein level is sometimes seen (Hughes et al, 1981).

8.4 Other biomedical (diagnostic) investigations and their interpretation

Electromyography, including single fibre electromyography (SFEMG) may be useful in differential diagnostics.

9. CLINICAL EFFECTS

9.1 Acute poisoning

9.1.1 Ingestion

Foodborne botulism

The initial symptoms may occur 18 to 36 hours post ingestion. They may be gastrointestinal especially in type E and include nausea, vomiting, abdominal cramps or diarrhoea. Constipation will predominate after the onset of neurological symptoms. The initial symptoms are dry mouth, blurring of vision and diplopia. These may be followed by ptosis, ophthalmoplegia, dysarthria, and dysphagia. These abnormalities of the cranial nerve are followed by a symmetrical descending pattern of weakness and paralysis. After the cranial nerves, the toxin affects the upper extremities, the respiratory muscles and, finally the lower extremities. If patients show signs of progression, they should be closely monitored for respiratory difficulties.

In severe cases, respiratory muscle paralysis may lead to ventilatory failure and death unless supportive care is provided (Shapiro et al, 1998). Ventilatory support may be required for long periods of time in severe cases (2 to 8 weeks). This increases the risk of medical complications. The recovery of autonomic function takes longer than that of neuromuscular transmission. Fatality rates are higher for older patients (greater than 60 years) and those who were index patients (the first patient in an outbreak), but antitoxin is effective in preventing progression of disease and in shortening the duration of ventilatory failure (Tacket, 1984).

Infant botulism

Infant botulism occurs in children of less than one year of age and mostly during the first 6 months of life. The clinical symptoms vary greatly from case to case. Constipation is frequently the first symptom, defined as 3 or more days without defecation. Progressive weakness and poor feeding follow after a few weeks. The weakness is symmetrical and descending as in foodborne botulism. It evolves over hours to several days. Other symptoms include lethargy, difficulty in sucking and swallowing, weak cry, hypotonia, pooled oral secretions and loss of head control.

Neurologic symptoms may include ptosis, ophthalmoplegia, weak gag reflex, dilated and sluggish pupils, dry mouth and neurogenic bladder.

The severity of the clinical picture varies from a mild intoxication to a fatal illness. However, prognosis is good with proper supportive treatment.

Adult infectious botulism

The clinical features of adult infectious botulism are similar to those of foodborne botulism except for the initial gastrointestinal symptomatology. The interval between bowel surgery or food exposure and the onset of clinical features may be one or more months. The clinical severity in reported cases has been quite variable.

9.1.2 Inhalation

Studies in monkeys indicate that, if aerosolised, botulinum toxin also can be absorbed through the lung (Shapiro et al, 1998).

9.1.3 Skin exposure

Neither *C. botulinum* spores nor its neurotoxins can be absorbed through intact skin, however damaged skin may be affected (Slovic & Jones 1998).

9.1.4 Eye contact

No data available.

9.1.5 Parenteral exposure

Wound botulism

Out of 40 published cases in the literature (Mechem & Walter, 1994) 78 % had a clear history of wounds, including abrasions, avulsions, lacerations, puncture wounds and abscesses. In other patients, the site of inoculation was obscure.

9.2 Chronic poisoning

9.2.1 Ingestion

Strictly speaking there is no such thing as chronic poisoning by botulism. In the cases of adult infectious botulism and infant botulism, the clinical picture may last several days or weeks. However, it is probably caused by a single exposure.

9.2.2 Inhalation

No data available.

9.2.3 Skin exposure

No data available.

9.2.4 Eye contact

No data available.

9.2.5 Parenteral exposure

No data available.

9.3 Course, prognosis, cause of death

Foodborne botulism

The symmetrical descending paralysis, when it occurs, usually appears 18 to 36 hours after exposure and generally lasts for 2 to 8 weeks. However, in severe cases, ventilatory support may be required for up to 7 months (Shapiro et al, 1998).

The prognosis is dependent on the quality of the supportive treatment. If adequate ventilation is maintained, the prognosis is good. If, however, ventilatory support is required for a long period of time (weeks to months) risks of medical complications (respiratory infections, ARDS) increase significantly. The improvement of critical care in recent years has reduced mortality from 50% to 9% (Cherington, 1998).

The cause of death in the first days following ingestion is respiratory failure due to a lack of adequate ventilatory support. In cases requiring long term ventilatory support, death is generally caused by medical complications.

Infant botulism

The course of the disease is extremely variable. Some are the fulminant type and difficult to differentiate from the Sudden Infant Death Syndrome (Midura, 1996). When the onset of illness is sufficiently gradual to permit hospitalisation, the prognosis is excellent.

Wound botulism

The prognosis for patients with wound botulism is favourable, assuming adequate ventilatory support is maintained (Mechem & Walter, 1994). The case-fatality rate for wound botulism is approximately 15 % (Shapiro et al, 1998).

9.4 Systematic description of clinical effects

9.4.1 Cardiovascular

Autonomic nervous system instability may induce tachycardia and hypertension. Orthostatic hypotension may also occur (Cherington 1998, Shapiro et al 1998).

9.4.2 Respiratory

Respiratory depression is caused by respiratory muscle paralysis. It may lead to ventilatory failure and death.

9.4.3 Neurological

9.4.3.1 CNS

Paralysis of cranial nerves causes blurred vision, diplopia, dysphonia, dysarthria and dysphagia.

9.4.3.2 Peripheral nervous system

Following the paralysis of the cranial nerves, a symmetrical descending paralysis will occur. It will affect the upper extremities, then the respiratory muscles, and, finally, the lower extremities in a proximal to distal manner.

9.4.3.3 Autonomic nervous system

Botulinum toxin causes a blockade of the autonomic cholinergic junctions resulting in dry mouth, blurred vision, orthostatic hypotension, constipation and urinary retention.

9.4.3.4 Skeletal and smooth muscle

Therapeutic use of botulinum toxin by direct injection of the drug produces a variety of histological changes (Montecucco et al, 1996). However, this has not been studied in cases of poisoning.

A case of gallbladder dysfunction induced by botulin A toxin has been described (Schnider P et al, 1993) as well as necrotising fasciitis as complication of botulinum toxin treatment (Latimer et al, 1998).

9.4.4 Gastrointestinal

Gastrointestinal symptoms may be observed 18 to 36 hours after ingestion in foodborne botulism. They include nausea, vomiting, abdominal cramps, and, occasionally, diarrhoea. In a later phase, after the onset of neurological symptoms and signs, constipation may occur. Gastric dilatation and paralytic ileus have been described (Adorjan et al, 1998).

Constipation is also frequently observed in infant botulism.

9.4.5 Hepatic

The liver is not affected by botulinum toxins.

9.4.6 Urinary

9.4.6.1 Renal

No direct effect.

9.4.6.2 Other

Neurogenic bladder may occur in the various forms of botulism.

9.4.7 Endocrine and reproductive systems

No direct effect.

9.4.8 Dermatological

No direct effect.

9.4.9 Eye, ear, nose, throat: local effects

Blurred vision, dysphagia, dry mouth, diplopia, dysarthria, ptosis, extraocular muscle weakness, reduced gag reflex, tongue weakness, fixed or dilated pupils, nystagmus may all be observed following the toxin induced blockade of cranial nerves and autonomic nervous system.

9.4.10 Haematological

No data available.

9.4.11 Immunological

Severe allergic reactions may occur following administration of the equine antitoxin.

9.4.12 Metabolic**9.4.12.1 Acid base disturbances**

Respiratory acidosis may occur if the ventilation is not properly supported.

9.4.12.2 Fluid and electrolyte disturbances

No data available.

9.4.12.3 Others

No data available.

9.4.13 Allergic reactions

None with the botulinum toxin but allergic reactions may occur with the administration of the equine antitoxin.

9.4.14 Other clinical effects

No data available.

9.4.15 Special risks*Foodborne botulism*

Home-canned and home-preserved food, uncured ham or sausages. Traditional food made with fish or sea-mammals.

Infant botulism

Ingestion of honey has implicated in some cases. Intestinal colonisation in adults has been associated with bowel surgery and chronic inflammatory disease of the intestine.

Inadvertent botulism

The therapeutic use of botulinum toxin needs special caution in patients with disturbed neuro-muscular transmission (myasthenia, Lambert-Eaton syndrome) and in patients concomitantly treated with aminoglycosides (Borodic, 1998; Wang et al, 1984).

A case report indicates that necrotising fasciitis can be a complication of botulinum toxin injection (Latimer et al, 1998).

9.5 Other

No data available.

10. MANAGEMENT**10.1 General principles**

Supportive treatment, especially adequate mechanical ventilation, is of prime importance in the management of severe botulism. Surgical debridement and antimicrobial treatment are also required in wound botulism. Antitoxin administration is the only specific pharmacological treatment available.

10.2 Life supportive procedures and symptomatic/specific treatment

Adequate mechanical ventilation is required following respiratory muscle paralysis caused by botulinum toxin. This may be required for a period of weeks or even months, especially in infant botulism. Special care should be taken in order to prevent secondary infections.

10.3 Decontamination*Foodborne botulism*

The efficacy of gastric decontamination in preventing botulism has not been studied. Since the features of foodborne botulism do not appear for several hours it is unlikely that gastric decontamination would be useful in an already symptomatic patient. In the case of recent ingestion (<1 hour) of possibly contaminated food emptying the stomach by induction of vomiting with syrup of ipecac, or by gastric lavage *could* be considered. Administer activated charcoal and a cathartic (such as sorbitol). Cathartic agents containing magnesium salts should be avoided because of the theoretical concern that increased magnesium levels may enhance the action of botulinum toxin (Shapiro, 1998).

Wound botulism

Surgical debridement should be performed.

10.4 Enhanced elimination

There is no way to increase the elimination of the toxin.

10.5 Antidote/antitoxin treatment

10.5.1 Adults

Foodborne botulism

One vial (7500 international units of type A, 5500 international units of type B and 8500 international units of type E antitoxins) equine antitoxin should be administered by infusion (Shapiro, 1998). Because of the risk of an allergic reaction to the equine serum, the patient should be asked about past history of asthma, hay fever or allergic reactions when in contact with horses.

Epinephrine chlorhydrate solution (1:1000) 1 mL should be available for immediate administration if required.

Sensitivity test:

An ocular or cutaneous sensitivity test should be performed prior to administration of the equine antitoxin.

Cutaneous test:

0.1 mL of the antitoxin serum diluted 1:100 in normal saline is administered by subcutaneous injection. If there is a positive history of allergies, this dose should be reduced to 0.05 mL of a 1:1000 dilution by subcutaneous injection. The interpretation of the result is done after 5 to 30 minutes. It is considered positive if a papule with a hyperemic areola occurs. The size of the papule and of the hyperemic zone give an indication of the level of sensitivity of the patient and the risk of an adverse effect to the administration of the antitoxin.

N.B. A negative cutaneous sensitivity test does not entirely exclude the possibility of a serum reaction.

Except in young children, an ocular test is easier to perform and produces less non-specific reactions. A drop of antitoxin serum diluted to 1:10 in a solution of normal saline is instilled in one eye. A control solution containing only normal saline is instilled in the other eye. Tears and conjunctivitis represent a positive reaction.

Serum reactions to equine antitoxin serums:

Anaphylactic reaction: Immediately administer 0.5 mL of a solution of epinephrine chlorhydrate 1:1000 SC or IM.

Fever:

This may occur 20 to 60 minutes after the administration of the antitoxin. It is characterised by shivering, slight dyspnea and fever.

Serum sickness:

This may occur up to 2 weeks after the administration of the antitoxin. The signs and symptoms are the following: fever, skin rash, oedema, swelling of the glands, articular pains. Urticarial reaction may respond to the administration of epinephrine. More severe cases may require the administration of cortisone.

Use of the equine antitoxin in a sensitive person.

Desensitisation protocol:

- 0.05 mL of a 1:20 dilution solution SC
- 0.1 mL of a 1:10 dilution solution SC
- 0.3 mL of a 1:10 dilution solution SC
- 0.1 mL of a non diluted solution
- 0.2 mL of a non diluted solution SC
- 0.5 mL of a non diluted solution SC
- Administration of the remaining therapeutic doses IM (Canadian Pharmacists Association, 1999)

Wound botulism

The treatment is similar to foodborne botulism.

Infant botulism

The use of equine antitoxin therapy is not recommended in children (Shapiro et al, 1998). However, the safety and efficacy of a human-derived antitoxin product (human botulism immune globulin) is being investigated in California (USA) for use in infants. For information on the Infant Botulism Prevention Programme contact the California Department of Health Services at (510) 540-2646 (24 hours).

Adult infectious botulism

The antitoxin protocol is the same as in foodborne poisoning. However, additional doses of antitoxin may be required. Care should be taken since sensitivity to equine serum may have been developed since the first administration.

10.5.2 Children

The protocol for the administration of the trivalent antitoxin is similar to the one used in adults.

11. ILLUSTRATIVE CASES

11.1 Case reports from literature

Wound botulism

A 27 year old heroin user was admitted with a 2 day history of muscle weakness. On examination he was afebrile and fully responsive but unable to keep his head upright. He deteriorated over the next few days, developing symmetrical flaccid paresis of the neck muscles, dysphagia, dysarthria, dry mouth, eyelid ptosis, mydriasis, diplopia, and urinary retention. He was unable to sit unsupported and had proximal paresis of all limbs with preserved deep tendon reflexes.

This patient usually administered heroin by subcutaneous or intramuscular injection and was noted to have several skin wounds. Wound botulism was suspected and he was treated with an intravenous dose of 500mL of trivalent equine botulism antitoxin (Botulism Antitoxin Behring, Chiron Behring, Germany) followed by 250mL six hours later. He was also given intravenous benzylpenicillin 20 megaunits daily, and surgical wound debridement was carried out. He developed respiratory failure and had to be mechanically ventilated for 16 days.

Electrophysiological investigations on day 26 revealed low compound muscle action potentials in the right arm and leg. Standard needle electromyography of the affected muscles showed brief low-amplitude irregular potentials. The diagnosis of wound botulism was confirmed in a mouse bioassay with serum drawn on day 3, just before administration of antitoxin (Jensenius et al, 2000).

Foodborne botulism

Six cases of botulism were described in Hungary. The first incident involved five members of the same family. The illness was moderately severe in three patients and mild in two patients. One of the patients had cirrhosis of the liver, and her condition became critical because of the repeated bleeding from oesophageal varices. A separate case involved a patient with sporadic illness. This patient developed severe gastric dilatation and paralysis of the bowels causing ileus at the start of the illness. In both sets of cases the diagnosis was confirmed by toxin tests in addition to the symptoms and food history. The symptoms regressed slowly, in about three weeks, in all patients. There were no deaths (Adorjan T et al, 1998).

12. ADDITIONAL INFORMATION

12.1 Specific preventive measures

C. botulinum produces heat-resistant spores. Some strains will not survive above 80C, but others can only be destroyed by heating above boiling point. The thermal resistance of spores increases in foods with a higher pH and a lower salt content (CDC, 1998).

The growth of *C. botulinum* is inhibited by high temperature, acidification, dehydration, salination, certain food preservatives e.g. nitrite, ascorbates, polyphosphates, and competing microorganisms such as *Lactobacillus* spp (CDC, 1998). Nitrite and nitrate food preservatives have their own inherent problems (WHO working group, 1977).

Botulinum toxin is heat labile and can be inactivated by heating to 80C (CDC, 1998).

The prevention of foodborne botulism is achieved by processing food in such a way as to kill spores, and/or inhibit bacterial growth, and/or denature preformed toxin. Since many cases of botulism are associated with home-preserved food, public education about the need for adequate heating, appropriate storage etc is important.

Since honey has been identified as a food source of infant botulism this food should not be given to infants under the age of one year.

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