Infant Botulism: Is There an Association With Thiamine Deficiency?

abstract

Infant botulism is an acute life-threatening condition and diagnosis is frequently delayed. Therefore, the best time window to administer specific antibodies, at present the only etiology-based therapy, is often missed, entailing long periods of hospitalization in the PICU. Here we present a 3-month-old boy with infant botulism and respiratory failure, who quickly and favorably responded to thiamine supplementation. From the feces we isolated *Clostridium botulinum* serotype A2. In addition to producing botulinum neurotoxin A, this strain carried the thiaminase I gene and produced thiaminase I. Accordingly, the child's feces were positive for thiaminase I activity. Because *C botulinum* group I strains are capable of producing thiaminase I, we speculate that thiamine degradation might further aggravate the paralytic symptoms caused by botulinum neurotoxins in infant botulism. Thus, supportive supplementation with thiamine could be beneficial to speed up recovery and to shorten hospitalization in some patients with infant botulism. *Pediatrics* 2014;134:e1436–e1440

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Infant botulism is an acute life-threatening condition that often leads to prolonged hospitalization in the PICU. Botulism is caused by botulinum neurotoxins (BoNTs) that are produced by *Clostridium botulinum* as well as strains of *Clostridium baratii* and *Clostridium butyricum*. BoNTs inhibit the release of acetylcholine from the presynaptic terminals of the neuromuscular endplate, block neuromuscular transmission, and cause flaccid muscle paralysis.[1,2] Thiamine is required as a cofactor for several metabolic processes and particularly for acetylcholine synthesis. Although the depletion of thiamine results in decreased acetylcholine production, botulinum toxin inhibits the release of acetylcholine from the presynaptic terminal. Both mechanism decrease the bioavailability of acetylcholine at the postsynaptic membrane of the neuromuscular junction. Therefore, infant botulism and thiamine deficiency may cause very similar neurologic symptoms in infants.[3–7] Of the 8 BoNT serotypes A through H, only serotypes A, B, E, F, and H cause human disease. There are 3 types of botulism: (1) the classic intoxication in food-borne botulism and (2) wound and (3) infant botulism.[1,8] Infant botulism results from toxin production in the bowels after intestinal colonization by *C botulinum*, when the normal competitive intestinal flora is disturbed or not yet established.[9] In the United States, infant botulism is the most common form, whereas in Europe, food-borne botulism is more prevalent. In most cases, the source of the spores remains elusive. Epidemiologic studies, however, established a link between infant botulism and honey consumption and in some cases the identical serotype was recovered from consumed honey and excreted feces.[10] Often, however, there is no history of honey ingestion and other relevant sources have to be explored (eg, household dust, infant formula).[11]

Typical symptoms of infant botulism are constipation, dysphagia, external ophthalmoplegia, and ptosis. Sucking reflex, pupil reaction, and facial expression are diminished or lacking and finally flaccid muscle paralysis leads to respiratory failure.[2] The infusion of antibodies against botulinum toxin is the only effective specific treatment but has to be administered within the first days of disease onset.[12,13] Antibiotic treatment is controversial. Because coinfection with *Clostridium difficile* has been reported as a cause of severe necrotizing enterocolitis, antibiotic treatment might be beneficial[14]; however, it might increase toxin levels and worsen the flaccid paralysis.[15]

PATIENT PRESENTATION

A normally developing 3-month-old boy was admitted to the hospital with a 7-day history of poor sucking, dehydration, sleepiness, muscle weakness, and constipation. He was exclusively breastfed and did not receive any honey or formula feeding. For breast care the mother had used biological healing wool (rinsed with rainwater only) and biological breast oil (containing D4 *Equisetum* [horsetail]). Ultrasound investigations of the child's abdomen and brain were normal. After 2 days of intravenous rehydration, progressive weakness, and impending respiratory insufficiency, the child was admitted to our PICU. Upon admission, he presented with tachypnea and paradoxical breathing. The chest radiograph revealed partial pulmonary atelectasis. Despite amimia and bilateral ptosis, he could open his eyes. His mydriatic pupils were light-reactive and tendon reflexes were normal. Twelve hours later, intermittent respiratory arrests and an increase in Pco_2 required noninvasive ventilation. The dilated pupils remained reactive to light, whereas the tendon reflexes had disappeared. Nerve conduction velocity was normal, including distal motor latencies as well as F-wave frequency and latency. The electromyogram did not show any decrement after repetitive stimulation suggestive of myasthenia or typical signs of botulism such as brief, small-amplitude, abundant motor-unit action potentials. Cerebrospinal fluid examination was normal, anti–acetylcholine-receptor antibodies were absent in the serum, and genetic screening for spinal muscular atrophy was negative.[16] Because of the disease course, we suspected infant botulism and sent blood and stool samples to the Robert Koch-Institute for analysis. At this time, specific antiserum treatment was no longer considered an option because of his complete paralysis and the presence of neurologic symptoms for >3 days.

Because severe thiamine deficiency (beriberi) or thiamine-dependent metabolic disorders may mimic botulism, we started thiamine supplementation with 100 mg/day.[3] Unfortunately, the blood sample for determination of the pretreatment thiamine level was lost on transport. To our surprise, the boy showed signs of recovery, such as movements of his upper limbs, 24 hours after the first thiamine dose. Oral supplementation was therefore maintained for 15 days while the child continuously improved.

The diagnosis of infant botulism was confirmed by a positive test for BoNT serotype A (BoNT/A) and the presence of BoNT/A-producing *C botulinum* species in the stools. In addition, *C difficile* toxin was found in the stools and we treated this coinfection with oral neomycin and intravenous metronidazole for 9 days to prevent necrotizing enterocolitis. The antibiotic treatment did not worsen the neurologic symptoms. After 5 days of treatment, all stool samples tested negative for BoNT and BoNT/A-producing *C botulinum* species. The child regained muscle strength and could be weaned off the ventilator after 18 days and discharged 12 days later.

METHODS AND RESULTS

A mouse bioassay identified BoNT/A in the stool extract.[17] The presence of BoNT/A was confirmed by a multiplex polymerase chain reaction (PCR) approach from stool samples as well as an anaerobic enrichment culture thereof.[18] Although the primary stool sample was positive for BoNT/A, samples taken after antibiotic treatment were negative, which indicates clearance of *C botulinum* from the gut. From the initial stool culture, we isolated a *C botulinum* group I strain named "Friedrichshain." In addition, *Clostridium perfringens* (strain RKI09057) but no *C difficile* was isolated. Sequencing of the 16S ribosomal RNA and *bont* genes was performed as described and identified a *bont/a* sequence of the Friedrichshain strain (GenBank HM051336), which belonged to the subtype BoNT/A2.[19]

In search for the source of infection with *C botulinum*, we tested household items with potential contact to our patient. Vacuum cleaner dust, pacifiers, breast oil, breast healing wool, and gardening gloves were subjected to anaerobic enrichment culture but all tested negative for *C botulinum*.

The speedy recovery soon after the first thiamine dose suggested a possible link between thiamine deficiency and the severity of neurologic symptoms in botulism. Literature research revealed that *Clostridium sporogenes* can produce thiaminase I, an enzyme capable of inactivating thiamine.[20] On the basis of morphology, 16S ribosomal RNA sequence and biochemical properties, group I strains of *C botulinum* are indistinguishable from *C sporogenes* except for the presence of the botulinum neurotoxin gene cluster.[21] We thus tested the isolated *C botulinum* strain Friedrichshain as well as the pretreatment stool samples for the presence of the thiaminase I (GenBank CP000728.1) gene. PCR with the use of the oligonucleotide primers (forward

oligonucleotide 5′-GAT TGT TAT AGT GAG GAT CCA CC-3′ and reverse oligonucleotide 5′-TAC TTT TTG TAT GTC ATA ATC TCC-3′) yielded the expected product of 246 bp. After antibiotic treatment, in parallel to the disappearance of the PCR signal for BoNT/A, the PCR product for thiaminase I had also vanished from the stool samples taken after antibiotic treatment. Because PCR does not necessarily prove the presence of the thiaminase I enzyme, we also determined thiaminase I activity as described.[22] Although no thiaminase I activity could be detected in stool extracts from healthy infant donors, the stool sample of the patient tested positive. Furthermore, the culture supernatant of the isolated *C botulinum* strain Friedrichshain revealed high levels of thiaminase I activity.

DISCUSSION

We present a case of infant botulism through intestinal colonization with the *C botulinum* strain Friedrichshain (serotype A2) that actively produced BoNT/A and thiaminase I. In the absence of honey ingestion, we searched for other sources of infection via household items but failed to identify the source of *C botulinum* spores. Although in some cases honey or household dust have been described as the source of spores, in most cases of infant botulism the exact source cannot be identified.[9]

For specific antibody treatment, only trivalent equine antibotulinum toxin (A, B, and E) hyperimmune globulin is readily available and licensed in Germany. Timely treatment is essential because cell-bound botulinum toxin can no longer be neutralized.[13] In our case, the delay of 48 hours between initial symptoms and paralysis with respiratory arrest, and thus the final diagnosis, had been too long to justify antiserum treatment. It has been reported that botulinum immunoglobulin intravenous (BIG-IV) sometimes might improve the clinical course even if

applied later than 24 to 72 hours after symptom onset; however, BIG-IV is not licensed in Germany. Untreated versus BIG-IV–treated infant botulism caused by *C botulinum* type A was associated with an average ventilator dependence of 6.4 (4.1–8.8) versus 2.0 (1.3–2.8) weeks, respectively.[13] Thus, weaning our patient, who had been treated with high-dose thiamine, off the ventilator after only 2.5 weeks can be considered a quick recovery.

Thiaminase I is found in most *C sporogenes* and *C botulinum* group I strains, but not in *C botulinum* group II strains.[20,23,24] Indeed, a Basic Local Alignment Search Tool search of the completed genomes of *C botulinum* in the National Center for Biotechnology Information database revealed that all group I but none of the group II strains harbor the thiaminase I gene. *C botulinum* consists of 4 groups (I–IV), which sufficiently differ by phylogenetic analysis and in their biochemical properties to be considered as 4 different species.[21] *C botulinum* group I strains include all 7 subtypes of serotype A (A1–A7), all but 1 subtype of serotype B (B1–B3, B5–B7), most subtypes of serotype F (F1–F5), and serotype H, whereas *C botulinum* group II strains include only subtype B4 of serotype B, most subtypes of serotype E (E1–E3, E6–E7), and subtype F6 of serotype F.[9,11]

In the randomized BIG-IV study by Arnon et al,[13] botulinum toxin type A was found in 60% and type B in 39% of the study patients. However, from the data published, it is not clear how many cases of infant botulism are caused by *C botulinum* group I and group II strains. *C botulinum* type B4 serotype cannot produce thiaminase I and therefore cannot cause thiamine deficiency. Although subtype B4 is frequently found in food-borne botulism cases in Europe, to our knowledge no case of infant botulism by this subtype has been reported.[9,11,13,23] Thus, it is likely that

almost all cases caused by serotype A and serotype B in Europe originate from *C botulinum* group I strains. This conclusion encourages us to suggest thiamine supplementation as a potentially beneficial factor in infant botulism because all *C botulinum* group I strains can produce thiaminases. Oral thiamine is safe, and intravenous thiamine administration has a very high safety profile as well. However, clinicians who use intravenous thiamine should be aware that, in rare cases, anaphylaxis has been reported.[25] Because the characterization of the botulinum neurotoxin and the *C botulinum* serotype is time consuming, it may unnecessarily delay the treatment with thiamine. Therefore, with respect to its safety and potential benefits, we would suggest an early administration of thiamine in case of suspected infant botulism, even if the laboratory analysis is not yet complete.

In infants, thiamine deficiency and infant botulism may have similar symptoms.[3] Thiamine is required for acetylcholine synthesis, whereas botulinum toxin inhibits its release. Both mechanisms add to the depletion of acetylcholine from the postsynaptic membrane, thereby blocking neuromuscular transmission.[4,6] Thiamine deficiency caused by intoxication with thiaminases is a well-known problem in grazing animals who may ingest high amounts of horsetail (*Equisetum*), bracken fern (*Pteridium aquilinum*), or nardoo (*Marsilea drummondii*).[26] The "anti-thiamine" effect of *Equisetum* was first described by Henderson et al[27] in horses. Thiaminases may be absorbed by the oral mucosa during rumination in cows and sheep or during intestinal passage in horses, causing profound thiamine deficiency. This thiamine deficiency leads to ataxia and flaccid muscle paralysis, which responds well to high-dose thiamine supplementation. The low amount of *Equisetum* in a homeopathic D4 dilution in the mother's breast oil was unlikely to be toxic for the child, because free thiaminases are inactivated by gastric juice. However, because *C botulinum* spores only germinate after gastric passage, thiaminases might well be absorbed by the intestinal mucosa in an alkaline milieu, leading to systemic thiamine depletion. One of the first human thiaminase poisonings on record was self-reported in the diaries of the British explorers Robert O'Hara Burke and William John Wills, who ran out of food during their exploration of Coopers Creek (Australia) in 1861 and started to chew on raw nardoo sporocarps. They were unaware that the Aborigines ate such sporocarps only after grinding and thorough cooking, which destroyed the thiaminases. The explorers eventually died an agonizing death from beriberi after 3 weeks, and their diaries were found much later.[28] A supportive effect of thiamine supplementation in patients suffering from botulism was first reported in 1953 by Henrich,[29] who described an unusually quick recovery in 2 adults suffering from food-borne botulism. In both patients, symptoms improved after the first 100-mg dose of intravenous thiamine.[29]

Even assuming the possibility of normal pretreatment thiamine blood levels in our patient, one could argue that, in general, supplementation with high doses of thiamine in patients with botulism might enhance clinical recovery by increasing acetylcholine synthesis and perhaps its bioavailability at the postsynaptic membrane.[29,30]

CONCLUSIONS

To our knowledge, this is the first report of biochemically proven thiaminase I activity in the feces of a child with infant botulism, who made an unusually speedy recovery after supplementation with thiamine. This single case draws attention to the fact that thiamine deficiency might be a complicating factor in infant botulism associated with *C botulinum* group I strains (serotypes A, B, F, and H). Hence, we suggest measuring serum thiamine levels in patients with suspected infant botulism and supplementation with thiamine if necessary. Future controlled studies will show whether thiamine supplementation has a beneficial effect on the outcome of infant botulism caused by *C botulinum* group I strains.

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