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Botulinum toxin infusion into the mesenteric artery has selective action on peristalsis in a rat model: experimental research

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Abstract. – OBJECTIVE: Botulinum toxin type A (BoNT/A) reversibly blocks neurotransmission at voluntary and autonomic cholinergic nerve terminals, inducing paralysis.

The aim of this study was to block panenteric peristalsis in rats through BoNT/A administration into the superior mesenteric artery (SMA) and to understand whether the toxin's action is selectively restricted to the perfused territory.

MATERIALS AND METHODS: Rats were infused through a 0.25-mm surgically inserted SMA catheter with different doses of BoNT/A (10 U, 20 U, 40 U BOTOX®, Allergan Inc.) or with saline for 24 h. Animals were free to move on an unrestricted diet. As a sign of bowel peristalsis impairment, body weight and oral/water intake were collected for 15 days. Statistical analysis was conducted with nonlinear mixed effects models to study the variation over time of the response variables.

In three 40 U-treated rats, the selectivity of the intra-arterial delivered toxin action was studied by examining bowel and voluntary muscle samples and checking the presence of BoNT/A-cleaved SNAP-25 (the smoking gun of the toxin action) using the Immunofluorescence (IF) method through a specific antibody recognition.

RESULTS: While control rats exhibited an increasing body weight, treated rats showed an initial dose-dependent weight reduction ($p<0.001$ control *vs.* treated) with recovery after Day 11 for 10 and 20 U-treated rats. Food and water intake over time showed significantly different half-saturation constants with rats treated with higher doses who reached half of the maximum achievable in a greater number of days ($p<0.0001$ control *vs.* treated rats). BoNT/A-cleaved SNAP-25 was identified in bowel wall NMJs and not in voluntary muscles, demonstrating the remarkable selectivity of arterially infused BoNT/A.

CONCLUSIONS: Blockade of intestinal peristalsis, can be induced in rats by slow infusion of BoNT/A into the SMA. The effect is long-lasting, dose-dependent and selective. BoNT/A delivery into the SMA through a percutaneous catheter could prove clinically useful in the treatment of entero-atmospheric fistula by temporarily reducing fistula output.

Key Words:
Botulinum toxin type A, SNAP-25, Superior mesenteric artery infusion, Intestinal peristalsis, Rat.

Introduction

Botulinum neurotoxin (BoNT) is a potent neurotoxin produced by anaerobic bacteria of the genus Clostridium. BoNT causes flaccid paralysis of striated and smooth muscle by inhibiting the release of acetylcholine (Ach) at the neuromuscular junctions (NMJs) in all mammalian species[1-3]. There are eight distinct serotypes of BoNT (BoNT/A- G and BoNT/X), according to their antigenicity[4-6]. Types A, B and E are commonly associated with botulism in humans[7].

BoNTs are made by 150-kDa protein organized into heavy chain (HC) and light chain (LC) domains linked by a disulphide bond. Since HC binds to presynaptic nerve terminals of the voluntary motor and autonomic NMJs, the disulphide bond is reduced and the LC is released into the cytosol where it cleaves serotype-specific protein (SNAP-25, VAMP, or syntaxin) of the SNARE complex. As this protein complex is required for the neuroexocytosis, presynaptic ACh release is blocked and muscle contraction is inhibited, resulting in a flaccid paralysis[8].

Among different serotypes, toxin type A (BoNT/A) is the most potent (followed by BoNT/B) and

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is used most frequently in clinical applications on striated muscle *via* intramuscular or subcutaneous injection, where its effects are evident both *in vitro* and *in vivo*[9-12]. After the Food and Drug Administration (FDA) approval of the use of BoNT/A in clinical practice in 1989, the drug was rapidly developed and applied in ophthalmology and neurology to treat strabismus, blepharon- and hemifacial- spasm[13]. At the same time, broader applications related to excessive muscle activity, such as cervical dystonia, spasmodic torticollis, and achalasia, have been explored[14-16]. BoNT/A injections are now widely used in plastic and reconstructive surgery for both aesthetic and non-aesthetic indications[17].

In the gastrointestinal tract (GI), ACh is considered the most important stimulating agent of the intrinsic (myoenteric) and extrinsic (vagal) nervous systems[18,19]. In case of adult foodborne BoNT intoxication, in addition to systemic muscle effects, the paralysis of intestinal musculature impairs peristalsis (with obstruction lasting for two or three weeks depending on the dose[20]) without permanent side effects when the motility function recovers[21]. In infant toxi-infection (where botulinum toxin is produced by *Clostridium* in the bowel), intestinal obstruction ensues and inhibits both intake of milk and passing of stools[22,23].

In experimental studies[24-27], BoNT/A injected directly into the GI wall was locally active on bowel musculature and gland secretions, which both depend on Ach release. BoNT/A has already been used for GI spastic disorders, such as achalasia, anal fissure, sphincter of Oddi dysfunction and other dyskinetic diseases (idiopathic gastroparesis)[28].

However, given the practical impossibility of addressing the entire bowel wall with multiple injections, a panenteric blockage of peristalsis has never been described so far.

The aim of this study was to block panenteric peristalsis in rats through BoNT/A administration into the superior mesenteric artery (SMA) and to understand whether the toxin's action is selectively restricted to the perfused territory.

Materials and Methods

Twenty-eight healthy, adult female/male Albino Wistar rats between 2 and 3 months of age with a mean weight of 560 g and aged 8-12 weeks were operated on. Each animal was anaesthetized with a mixture of ketamine and xylazine (100 mg/kg ketamine, 10 mg/kg xylazine) injected intramuscularly into an inferior paw. BoNT/A (BOTOX®, Allergan Inc, Campoverde di Aprilia, Italy) was infused into the SMA with the cannulation technique described in a previous study[29].

Prolonged, low-concentration perfusion was chosen to possibly improve the passage of the toxin through the intestinal capillary bed and reduce the risk of systemic intoxication. Accordingly, BoNT/A was infused in the first 24 h after surgery through a 5 mL/h Baxter® (Deerfield, Illinois, USA) elastomeric pump filled with 120 mL of saline where toxin was diluted and locked in a polystyrene box with ice inside to protect the toxin because of thermolability. The pump was then connected to the Silastic™ (Midland, Michigan, USA) cannula exiting from the back of the rat through a metal rod. During the infusion, the rats were free to move and eat. Once infusion ended, the external perfusion system was disassembled, and the rats were housed in a standard cage with a 12-h light/dark cycle with free access to food and water.

This study is reported in accordance with the ARRIVE guidelines (Animals in Research: Reporting *In Vivo* Experiments)[30].

Endpoints of the Study and Outcome Measures

The aims of the study were 1) to check the capacity of BoNT/A delivered through mesenteric artery, to realize panenteric peristaltic inhibition and 2) to study the selectivity of action of this regimen through the molecular identification of cleaved SNAP-25 (the target of BoNT/A) on both bowel and systemic NMJs.

Follow-Up

The twenty-eight rats subjected to the procedure were divided into 4 groups according to the BoNT/A administered dose: Group 1 was the control group of 8 animals that received only saline, Group 2 consisted of 3 animals that received 10 U of toxin, Group 3 consisted of 3 animals that received 20 U, and Group 4 consisted of 14 animals that received 40 U.

Food and water intake and body weight were measured daily during the entire observation period until sacrifice. Wellness animal indicators, such as behaviour, fur appearance, respiration (tachypnea/dyspnoea) and motions, were monitored daily to intercept eventual signs of systemic intoxication.

All rats were observed for 15 days, except those in Group 4. Group 4 animals showed signs of acute bowel obstruction from postoperative day (POD) 1 and were sacrificed on POD 4 according to the protocol and to minimize suffering.

At the end of the observation period, all animals were sacrificed. Specimens of the small bowel, diaphragm, and quadriceps femoris muscle were also collected in 3 rats from Group 4 for the molecular identification of cleaved SNAP-25.

Immunofluorescent Staining

All samples were processed as follows: bowel samples were peeled using small forceps to expose the myenteric plexus, and diaphragm and quadriceps femoris muscle samples were separated into bundles of approximately ten fibres. Tissues were quenched in 0.24% NH_4Cl phosphate-buffered saline (PBS) for 20 min. After permeabilization and 2 h of saturation in blocking solution (15% goat serum, 2% bovine serum albumin, 0.25% gelatine, 0.20% glycine, 0.5% Triton X-100 in PBS), tissues were incubated with homemade anti-cleaved SNAP25 primary antibody (1:500 in blocking solution) for 72 h at 4 °C. The anti-BoNT/A-cleaved SNAP-25 antibody specifically recognizes the cleaved form of SNAP-25 and not the whole protein (as demonstrated in Western blot experiments) both *in vitro* and *in vivo*[31-34].

Tissues were then washed in PBS and incubated with green, fluorescent αBungarotoxin (Invitrogen, Waltham, Massachusetts, USA) and Alexa Fluor 555-conjugated anti-rabbit secondary antibodies (Invitrogen, Waltham, Massachusetts, USA) for 2 h at room temperature and then mounted using Dako fluorescent mounting medium (Santa Clara, California, USA). Images were collected with a Leica SP5 confocal microscope (Wetzlar, Germany) equipped with a 40× HCX PL APA NA 1.4 oil immersion objective.

Statistical Analysis

Nonlinear mixed effects models were used to study the variation over time of the response variables (weight, food intake and water intake). For each individual unit *i*, the mean response depends on a nonlinear fashion by parameter vectors β_i, which may depend on the treatment group. See the **Appendix** for details.

A parabolic model was used to predict the increase or decrease in weight (in grams) over time following BoNT/A administration. Weight observations were normalized (at each time point, the observed weight was divided by the baseline weight) to exclude possible biases given by different initial rat weights. A Hill function was used instead to predict the saturated trend of food and water intake. For food and water modelling, data from group 4 were excluded by the fitting procedure since no data were collected after POD 4. The description of the models is reported in the Appendix. Models were fitted by maximizing the restricted log-likelihood (REML). The pairwise comparisons were assessed by post hoc analyses with Benjamin & Hochberg (1995) correction. Means and standard deviations were computed to summarize continuous variables. R software (Indianopolis, IN, USA) version 4.1.3, was used to perform all the statistical analyses. A p-value <0.05 was considered statistically significant.

Results

All rats survived surgery and toxin infusion. From POD 1, Group 1 (controls) showed an increasing body weight curve (only the growing branch of the parabola that opened upwards was therefore fitted). Conversely, Group 4 showed a decreasing trend, and only the decreasing branch of the parabola that opened downwards was adapted to the data. Groups 2 and 3 showed a decreasing trend during the first days up to the parabola vertex with a subsequent increase during the final days (Figure 1A). For these two groups, the weight recovery occurred at approximately in POD 11, which coincides with the vertex. Pairwise comparisons of parameter *b*, determining the steepness of the curve (due to the position of the parabola on the considered time range), between controls and treated rats were highly significant ($p<0.001$). Parameter *b* was significantly larger in Group 2 than in Group 3: the estimates were $b_{U10}= -0.018 \pm 0.005$ and $b_{U20}= -0.037 \pm 0.005$ ($p=0.017$). Smaller values of b (larger in absolute values) result in a faster weight loss during the initial phase. Parameters *a* and *c* were not significantly different among the groups.

For food and water intake, the effect of the toxin was found to be dose dependent, with the rats treated with 10 and 20 U showing a slow and progressive return to normal food and water intake in two weeks. Rats from the control group reached the maximum consumption of food more rapidly than rats treated with toxin. While the Hill coefficients of the food consumption curves were not significantly different between pairs of treatments (meaning that food consumption is

equally sensitive to the passing time), the half-saturation constants were found to be significantly different, with rats treated with higher dosages who reached half of the maximum achievable intake in a greater number of days.

Figure 1B shows the food consumption over time for each group (continuous lines for the predicted mean response curve, dots for the observations). Since rats from Group 4 were suppressed on POD 4, they were not included in the model fitting procedure, and the figure reports only the observed points. Estimates of the Hill coefficients were $y^{(0)}$ =2.53±0.35, $y^{(10)}$=2.34±0.31, and $y^{(20)}$=2.93±0.41 (p=0.682 for all comparisons). Parameters K were $K^{(0)}$= 1.99±0.42, $K^{(10)}$=5.35±0.74, and $K^{(20)}$=8.53±0.76 (p<0.001 for the comparison between the control group *vs.* treated groups; p=0.003 for the comparison between Groups 2 and 3). Parameter M was estimated at 31.47±0.64.

Water consumption curves resemble those for food. We determined estimates for parameter $y^{(g)}$, where $y^{(0)}$=3.59±0.47, $y^{(10)}$=3.11±0.38, $y^{(20)}$=2.23±0.24 (p=0.033 for the comparison between controls and Group 3). The values for parameter $K^{(g)}$ were $K^{(0)}$=1.73±0.13, $K^{(10)}$=4.70±0.27, and $K^{(20)}$=6.48±0.35 (p<0.001 for all comparisons). The estimate for parameter M was 48.37±0.62. Figure 1C shows the water consumption over time for each treatment group. Additionally, in this case, Group 4 was not considered in the fitting procedure. Table I reports the means and standard deviations corresponding to each treatment group and each time for weight and food and water consumption.

No rats showed signs of systemic intoxication: behaviour, fur appearance, respiration rate and motions appeared physiologic until the end of the observational period. The fur of the rats in Group 4 only looked faded on POD 4, probably due to severe malnutrition.

The immunofluorescent staining experiment, conducted on three rats from Group 4 (40 U of BoNT/A), showed that cleaved SNAP25 was uniformly present in the in neuronal processes of the myenteric plexus of all three intestinal samples (Figure 2A) and not in the NMJs of the diaphragm or quadriceps femoris samples (Figure 2 B-C), denoting BoNT/A proteolytic activity limited to the targeted tissue.

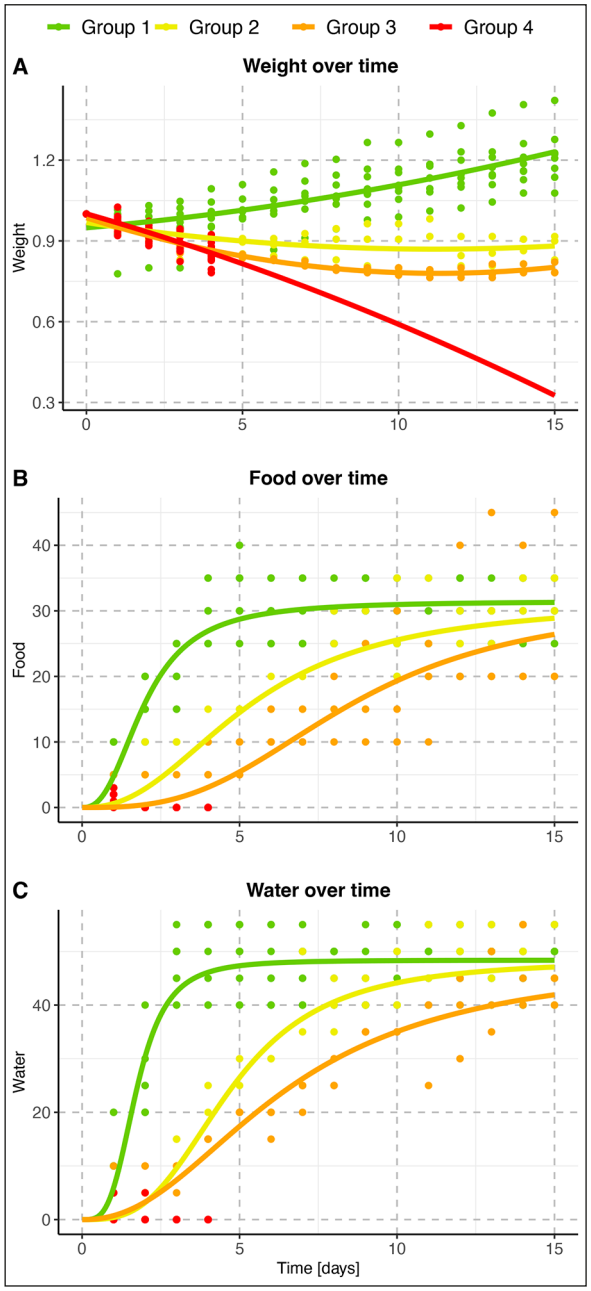

Figure 1. Weight (**A**), food consumption (**B**) and water consumption (**C**) over time for the four treatment groups. Continuous lines represent the predicted mean response curve from nonlinear mixed effects models; dots are used for observations.

Discussion

Even though botulinum toxin is widely used in the therapeutic inhibition of many voluntary and involuntary muscles (also in the GI tract)[28] and glands (as the parotid)[26,27] by topical injections, the dispersion area of each injected aliquot is approximately 1 cm^2, making the entire small bowel peristalsis block almost difficult to obtain[35,36]. Intra-arterial or intravenous administration of botulinum toxin, seldom reported as an adverse side effect during other BoNT/A procedures[37,38], has never been proposed with the aim of inducing

Table I. Means, standard deviations and number of studied rats corresponding to each treatment group and each observation time (number of days from toxicant administration).

Time (days)	Dose (group)	N	Weight Mean	SD	Food Mean	SD	Water Mean	SD
0	0 (1)	8	426.25	85.89	-	-	-	-
0	10 (2)	3	568.33	98.78	-	-	-	-
0	20 (3)	3	616.67	76.38	-	-	-	-
0	40 (4)	14	623.21	69.41	-	-	-	-
1	0 (1)	8	399.38	83.77	6.88	2.59	10.63	6.23
1	10 (2)	3	540.00	85.44	3.33	2.89	5.00	5.00
1	20 (3)	3	590.00	65.57	5.00	0.00	5.00	5.00
1	40 (4)	14	602.86	62.96	2.07	0.83	1.79	2.49
2	0 (1)	8	403.75	82.80	15.00	3.78	25.00	7.07
2	10 (2)	3	526.67	80.21	6.67	2.89	5.00	0.00
2	20 (3)	3	560.00	65.57	1.67	2.89	8.33	2.89
2	40 (4)	14	580.71	68.64	0.00	0.00	0.36	1.34
3	0 (1)	8	410.00	82.94	21.25	4.43	46.88	5.30
3	10 (2)	3	505.00	60.62	10.00	0.00	13.33	2.89
3	20 (3)	3	538.33	72.86	1.67	2.89	5.00	5.00
3	40 (4)	14	556.79	70.26	0.00	0.00	0.00	0.00
4	0 (1)	8	417.50	81.55	29.38	4.17	49.38	6.78
4	10 (2)	3	506.67	75.22	11.67	2.89	20.00	5.00
4	20 (3)	3	528.33	67.88	6.67	2.89	15.00	0.00
4	40 (4)	14	534.64	72.20	0.00	0.00	0.00	0.00
5	0 (1)	8	425.00	81.24	32.50	4.63	48.13	5.94
5	10 (2)	3	501.67	72.51	13.33	2.89	25.00	5.00
5	20 (3)	3	520.00	65.57	6.67	2.89	20.00	0.00
6	0 (1)	8	436.25	81.58	30.00	3.78	46.25	6.41
6	10 (2)	3	498.33	72.51	15.00	5.00	26.67	5.77
6	20 (3)	3	511.67	63.31	11.67	2.89	18.33	2.89
7	0 (1)	8	448.75	80.66	29.38	4.17	45.63	5.63
7	10 (2)	3	500.00	65.57	18.33	2.89	36.67	12.58
7	20 (3)	3	501.67	60.28	11.67	2.89	23.33	2.89
8	0 (1)	8	456.25	81.14	28.13	3.72	46.88	4.58
8	10 (2)	3	505.00	63.84	23.33	7.64	40.00	5.00
8	20 (3)	3	491.67	63.31	15.00	5.00	26.67	2.89
9	0 (1)	8	466.25	77.95	31.25	3.54	44.38	5.63
9	10 (2)	3	503.33	59.65	23.33	7.64	43.33	2.89
9	20 (3)	3	490.00	65.57	16.67	7.64	35.00	0.00
10	0 (1)	8	471.25	78.77	28.13	5.30	45.63	6.78
10	10 (2)	3	501.67	57.95	26.67	7.64	43.33	5.77
10	20 (3)	3	488.33	67.88	18.33	10.41	35.00	0.00
11	0 (1)	8	481.25	78.23	29.38	3.20	48.75	5.18
11	10 (2)	3	505.00	56.35	26.67	7.64	51.67	5.77
11	20 (3)	3	481.67	68.25	18.33	7.64	35.00	8.66
12	0 (1)	8	489.38	78.53	30.00	5.35	48.13	5.30
12	10 (2)	3	483.33	54.85	26.67	2.89	50.00	5.00
12	20 (3)	3	481.67	71.47	26.67	11.55	38.33	7.64
13	0 (1)	8	497.50	79.37	28.75	3.54	48.13	4.58
13	10 (2)	3	485.00	54.08	28.33	2.89	50.00	5.00
13	20 (3)	3	485.00	76.97	28.33	14.43	41.67	7.64
14	0 (1)	8	505.63	79.17	31.25	4.43	46.88	5.30
14	10 (2)	3	486.67	58.38	33.33	2.89	51.67	2.89
14	20 (3)	3	490.00	72.11	26.67	11.55	46.67	7.64
15	0 (1)	8	513.13	77.69	31.25	4.43	49.38	4.96
15	10 (2)	3	498.33	60.07	33.33	2.89	46.67	7.64
15	20 (3)	3	491.67	74.89	28.33	14.43	43.33	2.89

temporary panenteric reduction of peristalsis. The identification of an effective route to administer drugs in the arterial bed of the SMA during previous experiments[29] made us able to deliver BoN-T/A into the SMA by continuous slow infusion to explore the possibility of a panenteric paralysis.

Rats were chosen as the experimental model because they are animals with minimum neurological development (and a lower capacity to feel pain, distress, or prolonged damage) that are sufficiently susceptible to BoNT/A and have anatomy consistent with the surgical technique. SMA-functional cannulation can be performed through a proximal small branch of the SMA, which is irrelevant if closed, capable of allowing prolonged infusion without SMA thrombosis or leakage[29]. The main advantage of this infusion model was its steadfastness and durability for at least 24 h in all samples of the study.

Since botulism is dose-dependent[39], we hypothesized that a 24 h arterial continuous perfusion at low concentration would result in more efficient bowel NMJ intoxication and possibly in an elevated clearance of the toxin from blood in a single passage, so that systemic muscles remain unaffected.

Several serious adverse effects are described in the literature due to systemic venous spread of the toxin injected into the tissues, leading to botulism-like features[35]. They may include dysphagia, muscle weakness, allergic reactions[40,41] and, at worst, respiratory failure[42] with mechanical ventilation requirements. In this study, no adverse effects were observed after SMA infusion, and at the end of the observation period, no animals experienced respiratory or systemic symptoms; precocious sacrifice was required in 40 U administered rats because of severe bowel obstruction signs.

The hypothesis that BoNT/A infused into the SMA is cleared from the bloodstream during its first passage seems to be sustained by the evidence that cleaved SNAP-25 (with 9 amino acids cleaved from the terminal end) identified with anti-cleaved SNAP-25 specific antibody (BoNT/A footprint) is present only in the intestinal myoenteric plexus and not in other tissues downstream of the intestinal capillary bed, such as the diaphragm and the quadriceps femoris muscle.

Since botulinum toxin is absorbed in the bowel and reaches systemic muscles through the portal vein and the liver in foodborne intoxication, it is possible to conclude that the selectivity of action noted in this study after SMA infusion is not due to a supposed hepatic inactivation[43].

This is the first report both on the possibility of using arterial circulation to intoxicate organs with BoNT/A and the selectivity of this administration method. Tissue or organ intoxication via the arterial bloodstream could be an important improvement in the therapeutic use of botulinum toxin in humans. Currently, the clinical use of

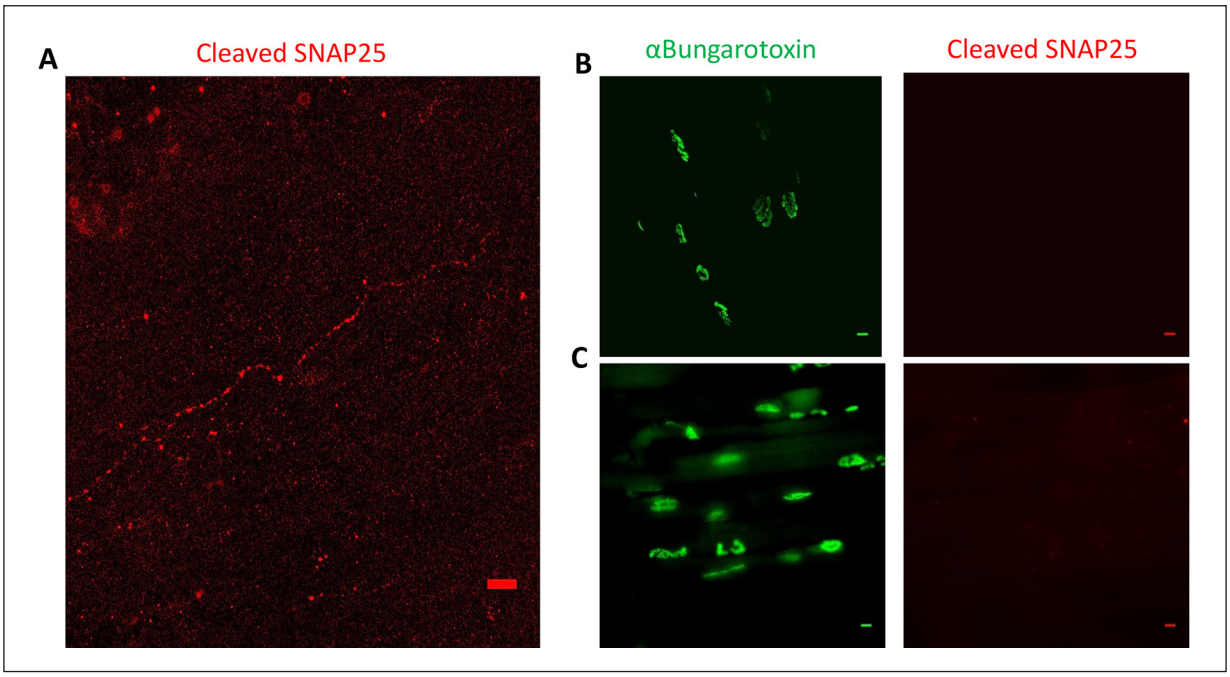

Figure 2. BoNT/A cleaves SNAP-25 only in myenteric neurons after mesenteric artery infusion. Representative immunostaining for BoNT/A proteolytic activity (highlighted in red with anti-cleaved SNAP 25 antibody) in neuronal processes of the myenteric plexus (**A**). The proteolytic activity of the toxin does not affect either diaphragm (**B**) or quadriceps femoris (**C**) neuromuscular junctions (highlighted with post-synaptic marker alfa-bungarotoxin in green), thus denoting a proteolytic activity limited to the targeted tissue. Scale bars: 15 μm.

the toxin is limited by the intratissutal injection method: if the organ is large (as leg muscles), many injections are necessary[44], and deeply located organs (as pancreas) are practically out of range[45]. The arterial administration could be performed in humans by radiologically assisted cannulation of the concerned vessel, particularly in the GI context where the botulinum toxin antisecretory activity has not been exploited thus far.

Our small series also represents a dose-finding experiment showing that while doses of 10 and 20 U are well tolerated by infused rats, 40 U is a near lethal dose causing acute, irreversible, and life-threatening intestinal occlusion.

Selective paralysis of the rat small bowel was a challenge between scientific demand and ethical rules: several precautions were undertaken to minimize animal suffering.

The limitation of this experimentation could be considered as the impossibility to study the long-term effect of the 40 U BoNT/A administered rats since sacrifice was necessary on POD 4 because of the drastic weight loss due to highly reduced food and water intake. Consequently, it was not possible to determine the recognized reversibility of BoNT/A action in the most intoxicated animals; its effect is temporary because the nervous cell is able to synthesize new SNAP-25 protein and revert neuromuscular transmission (in an animal model, the inhibition of neurotransmitter release by BoNT/A injected in tissues persisted for approximately 28 days[46]).

Moreover, another limitation is the exiguous number of cases observed: to be certain that clinical results, such as weight loss and reduced food and water intake, are associated with the molecular action of BoNT/A on the myenteric plexus, further molecular biology studies on tissue biopsies are necessary. A cross-check, which proves the presence of a SNAP-25 cleaved protein in intestinal smooth muscle but not in a diaphragmatic muscle or in a peripheral muscle in a bigger sample, would be statistically significant as evidence of the selective effect of intra-arterial administration of the toxin.

Prolonged inhibition of GI peristalsis could be clinically valuable in selected patients with enteroatmospheric fistula (EAF). EAFs are enteric fistulas occurring in the setting of an open abdomen that create communication between the GI tract and the external atmosphere[47]. EAF is a poor prognostic condition, frequently not amenable to surgery and rather challenging for management and nursing, so that mortality in such situations is reported to be as high as 42%[48].

The main problem of this condition is the continuous efflux of intestinal content and secretions in the open wound that impairs healing and creates a formidable burden for assistance despite vacuum-assisted systems for continuous removal of secretions. A reduced output of the enteric flow in the open wound, mediated by BoNT/A, could assist in preventing sepsis, in wound care and possibly in fistula closure.

Conclusions

Botulinum toxin has been rarely used in the gastrointestinal tract thus far. One of the reasons is the length and general extension of intra-abdominal organs, such as the small intestine or stomach, which cannot be treated with local infiltrations, or the difficulty of reaching these deep organs safely with a needle. However, the toxin is a therapeutic molecule capable of acting on both muscle contraction and glandular secretion with excellent safety characteristics and few side effects. The present study addresses this issue. After having described the possible route of administration in a previous stage, the toxin was infused directly into the superior mesenteric artery to verify the effects on the downstream territory. The toxin infused into the mesenteric artery reduces the intestinal functions in the rat in a selective way, and this was proven both by the clinical effects and by immunofluorescent staining, which show that it is present only at the intestinal level and not in other downstream tissues, such as the diaphragm and the leg muscles. The results of this study open up prospects for the therapeutic use of the toxin to selectively block intestinal peristalsis in the small bowel for a prolonged period in selected acute abdominal conditions. Infusion can be achieved in humans through the limited invasiveness of arterial catheterization.

Ethics Approval

Research procedures have been compliant with National Institutes of Health guidelines for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the local animal ethics committee (Catholic University of the Sacred Heart, Rome) and by the Italian Ministry of Health (protocol number: 237/2018).

Informed Consent

Not applicable.

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Data Availability
All data concerning to this study are reproducible and transparent. Since it is not possible to present all of the raw data in the article, we are available for sharing data upon request to the corresponding author.

Conflict of Interest
The authors declare no conflict of interest.

Authors' Contributions
Alessandro Borrello: Writing, Practical experiment, Methodology, Conceptualization, Investigation, Visualization. Amedea Agnes: Writing, Practical experiment, Methodology, Simona Panunzi: Data curation, Formal analysis. Ilaria Piergentili: Data curation, Formal analysis. Ornella Rossetto: Methodology, Conceptualization, Investigation, Visualization, Supervision. Federico Fabris: Practical experiment, Methodology, Conceptualization, Investigation, Visualization. Sabina Magalini: Writing - review & editing, Methodology, Conceptualization, Investigation, Visualization, Project administration, Data curation, Daniele Gui: Supervision, Writing - review & editing, Study design, Visualization, Methodology, Conceptualization, Investigation.

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