

Botulinum toxin injected in the gastric wall reduces body weight and food intake in rats

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SUMMARY

Background: Botulinum toxin is a powerful, long-acting inhibitor of muscular contractions in both voluntary and smooth muscle. It acts by blocking the release of the neurotransmitter acetylcholine. In the stomach, propulsive contractions of the antrum are necessary for the gastric contents to pass into the duodenum.

Aims: To investigate whether intramuscular injections of botulinum toxin type A into the gastric antrum of rats would cause a reduction in food intake and hence body weight, by inhibition of gastric emptying.

Materials and methods: This was a prospective, randomized, 3-way parallel group study in rats. The first group was anaesthetized, laparotomized and given 20 U of botulinum toxin type A by intramuscular injection into the gastric antrum (botulinum toxin type A group, $n = 14$). The second group was anaesthetized, laparotomized and injected with saline (sham group, $n = 14$) and the third group did not have any intervention (control group, $n = 5$). Food intake was measured daily for 7 weeks and body weight was measured daily for 10 weeks.

Results: There was a significant difference in loss of body weight between the two treated groups ($14.0 \pm 8.2\%$ botulinum toxin type A group, $4.4 \pm 2.7\%$ sham group; $P < 0.001$). Further, the time to reach the weight nadir was significantly longer in the botulinum toxin type A group (8.7 ± 3.9 days) compared with the sham group (5.3 ± 3.8 days; $P < 0.04$). There were no significant differences between the sham and control groups for any of the body weight parameters. The minimum dietary intake was significantly lower in the botulinum toxin type A group than in the sham group ($37.8 \pm 21.8\%$ of the basal value in the botulinum toxin type A group, vs. 65.5 ± 32.0 in the sham group, $P < 0.05$). In addition, the time to reach the nadir was significantly prolonged (8.2 ± 3.5 days, botulinum toxin type A group vs. 4.9 ± 1.7 days, sham group, $P < 0.001$).

Conclusions: The parallel reduction of body weight and food intake in botulinum toxin type A treated animals is consistent with a long lasting inhibition of the antral pump. This is probably due to slowed gastric emptying leading to early satiety. Patients with morbid obesity might benefit from endoscopic injections of botulinum toxin type A into the stomach wall.

INTRODUCTION

Botulinum toxin is produced by the bacterium *Clostridium botulinum* in seven serotypes (A through G). It is the

most powerful known inhibitor of muscular contraction and acts by interfering with acetylcholine release in the neuromuscular plaque.^{1, 2} When ingested, botulinum toxin can lead to the form of food poisoning known as botulism. However, it is also a highly effective medication in patients with neural and muscular disorders. When the toxin is injected directly into muscle, the

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resultant inhibition of muscle contraction is selective, prolonged and reversible, without significant adverse effects. Since 1973, botulinum toxin type A has been successfully used in the treatment of voluntary muscle contraction disorders, such as strabismus, dystonia and tremors.^{3, 4}

In the gastrointestinal tract, several chemical mediators have excitatory or inhibitory properties on peristalsis, but the neurotransmitter acetylcholine is generally considered the most important stimulating agent both in the intrinsic (myoenteric) and the extrinsic nervous system^{5, 6}. In the stomach, propulsive contractions of the antrum, which are necessary for the gastric contents to pass into the duodenum, are mediated by vagus nerve stimulation. We therefore investigated the effect of botulinum toxin type A on food intake and body weight in rats by injecting botulinum toxin type A into the muscular wall of the antrum, thereby inhibiting the motility of the antral pump.

MATERIALS AND METHODS

Experimental procedure

Fourteen adult Wistar rats (weight 500–700 g) were anaesthetized with intraperitoneal injections of diazepam (2 mg/kg) and ketamine (2 mg/kg). A short abdominal incision was made and the antrum of the stomach was isolated. The rats were injected with botulinum toxin type A (Botox; Allergan) using a 27G needle. A total of 20 U of the toxin were diluted in 1 mL saline and injected subserosally into the four cardinal points of the antrum: on the small and large curve, and on the posterior and anterior wall (5 U in each 0.25 mL injection, botulinum toxin type A group). A further 14 rats were anaesthetized and laparotomized, but injected with saline (sham group). Five more rats did not receive any intervention (control group).

The day of operation for the rats in the botulinum toxin type A and sham groups was taken as day 0. In order to follow natural growth in the control group, an arbitrary initial day of observation was designated day 0.

The body weights of all rats were measured and recorded every morning from day 0 until the 10th week. The weight of daily ingested food was measured and recorded from day 0 until the 7th week. Rats were allowed to feed *ad libitum* from day 1 onwards, with standard dry pellet diet.

An additional small group of rats (two treated with botulinum toxin type A as described above and two with saline) was fed with a mixture of iron filings and cheese from 10 days after their operation.

Modelling and statistics

A simple parametric model of the process of weight decrease and successive weight gain of a rat subjected to surgery has been used. With this model, the rat is assumed to experience an exponential decay of its weight from day 0 until the nadir. After the nadir, the animal is assumed to regain weight at an approximately constant rate.

Taking the weight of the rat at the nadir as

$$W_n = W_0 e^{-K_d t_n} \quad (1)$$

the weight of the animal as a function of time may be expressed as

$$w(t) = W_0 e^{-K_d t}, \quad 0 \leq t \leq T_n \text{ and} \quad (2)$$

$$w(t) = W_n + G_r(t - T_n), \quad t > T_n \quad (3)$$

in the weight decrease (Eq. 2) and weight increase (Eq. 3) phases, respectively, where

w [g] is the weight of the rat as a function of time;

W_n [g] is the weight of the rat at the nadir (lowest predicted weight after surgery);

W_0 [g] is the initial weight of the animal;

K_d [day⁻¹] is the exponential decrease rate constant for the first-phase loss of weight;

t [day] is the time;

T_n [day] is the time needed to reach the nadir weight;

G_r [g/day] is the rate of weight gain in the second phase.

Observations of time and weight on each rat were fitted by means of a non-linear least squares, quasi-Newton algorithm, to yield estimated values of the model's three free parameters K_d , T_n and G_r ⁴. From the free parameter values, the value of the predicted nadir weight (W_n) of the maximal weight drop ($W_0 - W_n$) and of the maximal weight drop expressed as a percentage of the starting weight ($100(W_0 - W_n)/W_0$) were calculated for each rat. An analysis of variance with simple contrasts was conducted on the parameters relative to each rat, in order to evaluate the significance of the overall difference between the means of the three groups

(botulinum toxin type A, sham and control), the significance of the difference between sham operation and no operation at all (sham vs. control) and the significance of the difference between toxin treatment and sham operation (botulinum toxin type A vs. sham).

A Mann–Whitney *U*-test for comparison of independent non-parametric samples was conducted between the botulinum toxin type A and sham groups for minimum post-operative diet intake (nadir, expressed as a percentage of the average basal diet intake) and for the number of days needed to reach the nadir.

RESULTS

One of the rats in the sham group died at the end of surgery. No other untoward events were recorded for any of the other rats who underwent operations.

The sample averages and standard deviations of the estimated free and derived model parameter values for body weight are given in Table 1. The significance levels of the overall differences between the three treatment groups, the difference between the sham group and the control group, and the difference between the botulinum toxin type A group and sham group are also given in Table 1. The starting weights and the standard errors of the estimates in the non-linear regression are also presented in this table.

The starting weight of the animals was not significantly different between the three groups, being 600, 562 and 599 g in the control, sham and botulinum

toxin type A groups, respectively. The body weight of the rats who underwent an operation dropped after surgery, but eventually returned to baseline values. The fit of the model to the experimental data, as measured by the standard error of the estimate, was approximately the same in all of the groups, indicating that the data-points of none of the groups were significantly better (or worse) than the other groups.

There was a significant ($P < 0.001$) reduction in mean weight and in the maximum percentage weight reduction ($P < 0.001$), in the botulinum toxin type A group compared with the sham group. The differences between the sham and control groups were not significant. The time to reach the weight nadir was significantly longer in the botulinum toxin type A group compared with the sham group ($P < 0.04$), while the rate of weight gain after the nadir was not significantly different between the three treatment groups. Figure 1 illustrates the time-course of the weight of the animals from day 0, averaged for the three groups and expressed as a percentage of the group average initial weights. Inspection of the figure reveals that the reduction in weight in the botulinum toxin type A group compared with the sham group was prolonged and sustained over the first 7 weeks of the study. After this time, the number of data points is too low for inferences to be drawn.

Table 2 gives the values of food intake for rats in the sham and botulinum toxin type A groups. The basal weights of the daily ingested dry food (as an average of

Table 1. Sample averages and standard deviations of the body weight parameters. Full details of the derivation of the body weight parameters are given in the text. P_{overall} gives the significance level of the overall difference between the means of the three groups; P_{surgery} is the significance level of the difference between the sham and control groups and P_{toxin} is the significance level of the difference between the sham and botulinum toxin type A groups

Variable	Non-operated controls (control: $n = 5$) (Btx: $N = 14$)	Sham operation (sham: $n = 13$)	Toxin operation	P_{overall}	P_{surgery} Sham/C	P_{toxin} Btx/Sham
Starting weight [g]	600.0 $\pm$ 54.88	562.0 $\pm$ 55.24	599.0 $\pm$ 52.29	N.S.	N.S.	N.S.
K_d : growth decrement rate constant [day ⁻¹]	0.0039 $\pm$ 0.0063	0.0166 $\pm$ 0.0190	0.0184 $\pm$ 0.0110	N.S.	N.S.	N.S.
T_n : time to nadir [day]	4.072 $\pm$ 5.049	5.327 $\pm$ 3.800	8.659 $\pm$ 3.878	0.045	N.S.	0.040
G_r : growth rate [g/day]	0.0776 $\pm$ 0.1009	0.5821 $\pm$ 0.7666	0.9461 $\pm$ 0.7017	N.S.	N.S.	N.S.
Maximal weight drop [g]	6.523 $\pm$ 8.573	25.63 $\pm$ 17.15	83.98 $\pm$ 47.73	< 0.001	N.S.	< 0.001
Maximal weight drop as percentage of starting weight (%)	1.007 $\pm$ 1.246	4.366 $\pm$ 2.708	14.07 $\pm$ 8.150	< 0.001	N.S.	< 0.001
Standard error of estimate [g]	12.49 $\pm$ 5.850	12.39 $\pm$ 9.088	12.46 $\pm$ 8.309	N.S.	N.S.	N.S.

Btx = botulinum toxin type A.

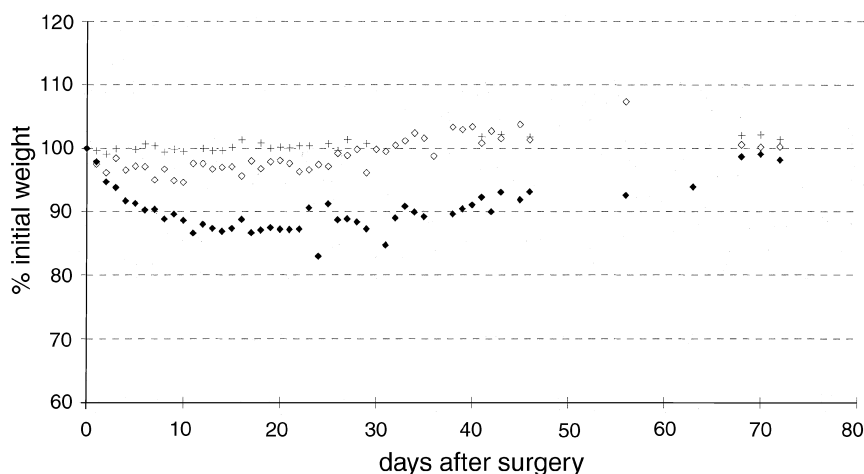


Figure 1. Time courses of rat body weights averaged by group and expressed as a percentage of group-averaged starting weight: +, control group; ◇, sham group, ◆, botulinum toxin type A group.

Variable	Sham operation (sham: $n = 13$)	Toxin operation (btx A: $n = 14$)	P
Basal food intake [g]	30.1 ± 2.5	32.4 ± 3.9	N.S.
Time to nadir [day]	4.9 ± 1.66	8.2 ± 3.53	< 0.001
Minimum daily intake as percentage of basal [%]	65.5 ± 32.0	37.8 ± 21.8	< 0.05

Table 2. Sample averages and standard deviations of daily food intake, time to nadir and minimum daily intake in the sham and botulinum toxin type A treated rats. The P -values express the significance level of the difference between the sham and botulinum toxin type A groups

3 days prior to operation) were 30.1 and 32.4 g in the sham and botulinum toxin type A groups, respectively. Dietary intake at the nadir, expressed as a percentage of the mean pre-surgery value, was significantly ($P < 0.05$) smaller in the botulinum toxin type A group compared with the sham group. The time to reach the nadir was also significantly ($P < 0.001$) prolonged in the botulinum toxin type A group compared with the sham group.

Figure 2 illustrates the average time course of the daily food intakes in the botulinum toxin type A and sham groups. Daily food intake decreased after surgery and subsequently increased in both groups, although rats in the botulinum toxin type A group showed a more pronounced and longer-lasting reduced intake compared with the sham group.

DISCUSSION

Neurotoxins produced by *Clostridium botulinum* bind with high affinity to peripheral cholinergic nerve endings, such as those at the neuromuscular junction, and in the autonomic nervous system. Each of the seven toxin serotypes is synthesized as a single-chain polypeptide (molecular weight, 150 000) with minimal toxicity.¹ The single-chain molecule is cleaved by

proteolysis and generates a heavy chain (molecular weight, 100 000) that is linked by a disulphide bond to a light chain (molecular weight, 50 000). The dichain form of the molecule is taken up by the nerve endings of cholinergic fibers and, through a complex sequence of events, blocks the 'fusion machine' which allows the exocytosis of neuromediators in the neuromuscular plaque.⁷ The active part of the toxin is the light chain, which acts as a zinc-dependent endoprotease in the cytosol. Of the seven different botulinum toxin (botox) serotypes, the A, C and E types have SNAP-25 as the molecular target. Under the action of the administered botox type A, a nine aminoacid fragment is removed from the carboxiterminal region of this fusion membrane protein, thus inactivating it.

In clinical applications, dystonic muscles are periodically injected with small amounts of botulinum toxin type A, leading to reduced strength of contraction or even to complete paralysis, depending on the dose. Botulinum toxin type A is currently considered the treatment of choice for strabismus, blepharospasm, torticollis and dystonia. Botulinum toxin serotype A toxin is highly selective for cholinergic cells (decreasing the opportunity for adverse side-effects) and has a long duration of action. Clinically significant responses can last from 1 to several months, after which time the toxin

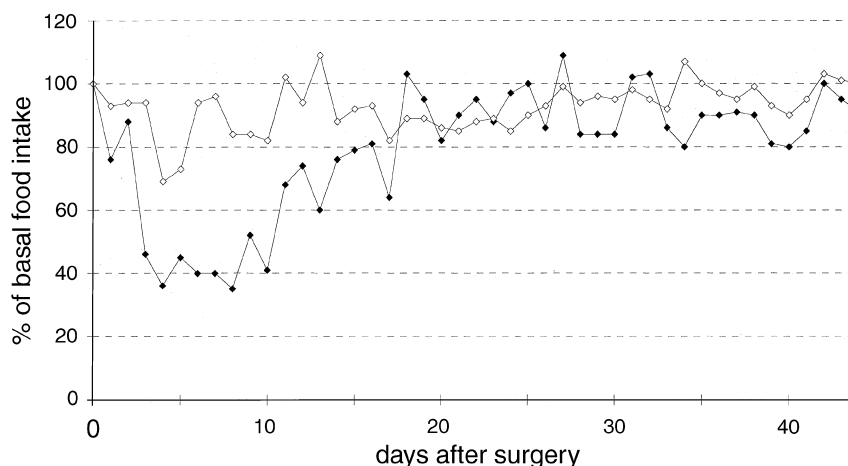


Figure 2. Time courses of daily food intakes averaged by group and expressed as percentage of basal food intake: $\diamond$, sham group; $\blacklozenge$ botulinum toxin type A group.

must be re-administered. Finally, the dose of toxin can be individually titrated for each patient to ensure maximal benefits.³

To date, few experimental or clinical trials have been conducted to investigate the effects of botulinum toxin type A on the smooth muscle of the gastrointestinal tract.^{8,9} In 1993, Parischa *et al.* demonstrated an inhibitory effect on lower oesophageal sphincter tone in pigs.¹⁰ They then successfully treated some high risk achalasia patients by injecting the sphincter by endoscopy.¹¹ In 1994 we demonstrated that botulinum toxin type A reduces the strength of the smooth (internal) anal sphincter when injected into the muscle, leading to healing of anal fissures, which are pathogenically linked to sphincter hypertonus.¹²

Gastrointestinal motility encompasses a number of events: myogenic events in the muscular cell (myocyte) leading to spontaneous contraction; neurogenic events by both intrinsic and extrinsic nerves; and coordinated contractions which lead to elevated intraluminal pressure and propulsion of contents. Spontaneous contraction is effected without the action of neuromediators, depolarization being communicated from cell to cell through connecting gap junctions. However, the intrinsic (myoenteric) nervous system is the major controller of intrinsic motility.⁵ Neurotransmitters are cholinergic (stimulatory), adrenergic (inhibitory) and non-adrenergic non-cholinergic (the physiological roles of these last are largely unknown). The extrinsic nervous system modulates the myoenteric system through excitatory (parasympathetic) and inhibitory (sympathetic) pathways. Even though several neuromediators are present, motility is mostly dependent on the neurotransmitter acetylcholine.

In the stomach, extrinsic vagal innervation mediates postprandial induction of strong antral contractions and simultaneous relaxation of the fundus, shearing solid food and differentially delivering solid and liquid contents into the duodenum. The proximal stomach (fundus and oral third of the corpus) exerts a steady pressure on gastric contents by sustained contraction and plays a major role in gastric emptying of liquids. In contrast, the distal stomach (two thirds of the corpus and the antrum) controls the emptying of solids.

Interdigestive migrating motor complexes, which originate in the antrum and migrate caudally through most of the small bowel, are the main organized peristaltic waves. Rings of contraction sweep distally through the distal antrum, the strongest ones occluding the gastric lumen and propelling chyme through the pylorus into the duodenum.

In accordance with above description, botulinum toxin injected into the muscular wall of the antrum should interfere selectively with gastric emptying of solid food by reducing the strength of peristaltic waves, the emptying of liquids being unaffected by antrum motility. In addition, myogenic motility events should not be affected because they do not use mediators, nor should the propagation of peristalsis through the small bowel be affected because it is independent of the strength of muscle contraction.

In the study reported here, the reduction in body weight and food intake demonstrated in botulinum toxin type A treated rats is consistent with a strong inhibitory effect on gastric emptying of solid food. No treated animals died of dehydration throughout the experimental period, which is consistent with the free passage of fluid contents from the stomach. It is also of

note that the duration of effect of a single treatment with botulinum toxin type A was similar to that observed in humans.

The possibility should be considered that injection of botulinum toxin type A into the antrum could be of benefit in patients with morbid obesity. Moderate to severe obesity is a common condition, affecting 10% of people in the USA, and is associated with many metabolic diseases (diabetes, hypertension, etc.) leading to a reduced life span. Morbid obesity is usually related to a chronically high calorie intake. However, despite many efforts in world-wide research, in a large number of patients, weight reduction cannot be accomplished and maintained by diet alone, or by drugs that decrease food craving.

Among the therapeutic options for morbid obesity is surgery: gastric restriction produces early satiety which then affects oral feeding. In the past, several attempts have been made to reduce gastric compliance by non-invasive means. A semi-permanent balloon has been inserted through the oesophagus and inflated in the stomach, but with very poor clinical results. The rationale of slowing gastric emptying by the use of drugs has not been studied due to the lack of an appropriate chemical tool.

It is suggested that lowering the rate of gastric emptying by the use of botulinum toxin type A could be easily and repeatedly accomplished in obese patients, with minimally invasive intervention via the endoscopic route. As gastrointestinal motility seems to be normal in obesity, the effect of the toxin should be quite similar to that described in the present work: an early feeling of satiety should ensue, leading to a reduction in food intake.^{13, 14} The temporary action of the toxin could be of special value in severely obese patients when rapid weight loss is needed in order to reduce post-operative risk. Further studies are required.

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