

Botulinum neurotoxin to treat chronic anal fissure: results of a randomized 'Botox vs. Dysport' controlled trial

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SUMMARY

Background: Botulinum neurotoxin induces healing in patients with idiopathic fissure. The optimal dosage is not well established.

Aim: To compare the efficacy and tolerability of two different formulations of type A botulinum neurotoxin, and to provide more evidence with regard to the choice of dosage regimens.

Methods: Symptomatic adults with chronic anal fissure were enrolled in a randomized study. The outcome of each group was evaluated clinically, and by comparing the pressure of the anal sphincters before and after treatment.

Results: Fifty patients received injections of 50 units of Botox formulation (group I), and 50 patients received injections of 150 units of Dysport toxin (group II). One

month after injection, 11 patients in group I and eight in group II had mild incontinence of flatus. At the 2-month evaluation point, 46 patients in group I and 47 patients in group II had a healing scar. In group I patients, the mean resting anal pressure was 41.8% lower, and the maximum voluntary squeeze pressure was 20.2% lower, than the baseline value. In group II patients, the resting anal pressure and maximum voluntary squeeze pressure were 60.0 ± 12.0 mmHg and 71.0 ± 30.0 mmHg, respectively. There were no relapses during an average of about 21 months of follow-up.

Conclusions: Botulinum neurotoxin may be considered an effective treatment in patients with chronic anal fissure. The efficacy and tolerability of the two different formulations of botulinum neurotoxin were indistinguishable.

INTRODUCTION

Anal fissure is a distressing condition. The aetiology of chronic anal fissure remains controversial, although spasm of the internal anal sphincter has been recognized to play a central role in the pathogenesis of the disease. Surgical sphincterotomy, which is widely performed to provide symptomatic relief and healing, is highly effective, but can be associated with permanent complications.^{1–3}

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Chemical denervation with type A botulinum neurotoxin has been proposed for the treatment of this condition without any risk of permanent internal anal sphincter injury. This is a versatile tool for targeted weakening of smooth muscle in the gastrointestinal tract and, compared with other indications, such as oesophageal achalasia, the effect is not limited to symptomatic improvement alone, but also produces healing in the majority of patients.^{4–8}

Two preparations of serotype A of botulinum neurotoxin are available for clinical use: the European preparation (Dysport; Speywood Biopharm Ltd, Wrexham, UK) and the American preparation (Botox; Allergan, Irvine, CA, USA). Unfortunately, there has

been much confusion over the doses and units of potency of the two preparations. Although doses are quoted in mouse units (which is the amount of toxin that kills 50% of a group of 18–20-g female Swiss-Webster mice), implying some standardization, Botox seems to be more potent. It is suspected that 1 Botox unit is approximately equivalent to 3–5 Dysport units. Furthermore, a Dysport/Botox equivalency ratio of approximately 3 : 1 or 5 : 1 at standard vial dilutions has been suggested.

Lack of awareness of this difference may have disastrous consequences in clinical practice. If there is no correction of the respective doses, patients may experience either effects several times more intense than usual if they are switched from Dysport to Botox, or failed treatment if they are transferred from Botox to Dysport. This has implications with regard to safety as well as cost, as the treatments are expensive and need to be optimized.

In patients with chronic anal fissure, the optimal dose of botulinum neurotoxin is unclear.^{9, 10} The injection of 20 units with a 27-gauge needle into the internal sphincter on each side of the fissure has been reported to be optimal.¹¹ In one study, anterior injection of botulinum toxin was associated with earlier healing of a posterior fissure than was posterior injection.¹² We have conducted a prospective, randomized trial of the two formulations (Botox and Dysport) to test the hypothesis that, at a conversion factor of 1 : 3, the clinical effects are indistinguishable. The objective was to compare the efficacy and tolerability of the two different formulations of type A botulinum neurotoxin injected into the anterior site of the internal anal sphincter, and to provide more evidence with regard to the choice of dosage regimens (50 Botox units or 150 Dysport units; the conversion factor of 1 : 3 was chosen on the basis of previous reports in the literature). The long-term results and complications in both groups of patients were also evaluated.

MATERIALS AND METHODS

Study patients

Consecutive symptomatic adults with chronic anal fissure were enrolled in the study. The diagnosis was based on the following clinical criteria: (i) evidence of

posterior circumscribed ulcer with a large sentinel tag of skin, induration at the edges and exposure of the horizontal fibres of the internal anal sphincter; and (ii) symptoms (post-defecation and/or nocturnal pain, bleeding) persistently present for over 2 months.

The exclusion criteria were as follows: acute fissure; anal fissure associated with different pathologies (i.e. inflammatory bowel disease, human immunodeficiency virus infection, haemorrhoids, fistula-in-ano, anal abscesses, anal or perianal cancer); previous surgical procedures on the anal canal; the existence of systemic diseases that could confound the evaluations; concomitant oral medications that could interfere with the action of type A botulinum neurotoxin, such as aminoglycosides, baclofen, dantrolene or diazepam; known hypersensitivity to any of the components of the formulations of type A botulinum neurotoxin; pregnant or breast-feeding women.

The study protocol was reviewed and approved by the Institutional Committee of the Catholic University of Rome. Each patient provided written informed consent to the study.

Study design

From January to December 2001, eligible patients were enrolled in the study. Randomization was performed after informed consent had been obtained. The randomization schedule was generated by computer. Allocations were sealed in opaque numbered envelopes, opened after collection of the baseline data. Randomization was not available to the investigators. The code was broken at 2 months when the patients had completed the first part of the trial. At 1 and 2 months of follow-up, one examiner, blind to the nature of the type A botulinum neurotoxin formulation used, evaluated the outcome. The outcome of each group was evaluated clinically, and by comparing the pressure of the anal sphincters, as measured by anorectal manometry, before and after treatment.

The study end-point was the evaluation of complete healing after type A botulinum toxin injection. The treatment was considered to be successful if the fissure healed. Persistence of the fissure in the absence of symptoms was considered as symptomatic improvement. A secondary variable was the latency of the effect, defined as the interval between the day of treatment and the start of symptomatic relief.

Baseline assessment and operative technique

All patients underwent a pre-treatment evaluation, including clinical inspection and anorectal manometry. Anorectal manometry was performed at rest and after maximum voluntary contraction, and was compared with the normal range for our laboratory, as reported elsewhere^{4,9}. The resting anal tone and maximal squeeze pressure (i.e. the maximal voluntary increase above the resting tone) were measured according to a stationary pull-through technique. One and two months after treatment, patients underwent the same evaluation as performed at baseline.

Type A botulinum neurotoxin was diluted in saline. The internal anal sphincter was palpated and injected with a 27-gauge needle, with the patient lying on the left side (Figure 1). Neither sedation nor local anaesthesia was used during the procedure. After baseline evaluation, each of the participants received 1 mL of solution injected into the internal anal sphincter on each side of the anterior midline, divided into two injections of equal volume (0.5 mL).

Clinical care, follow-up and outcome measures

No patient was treated with topical anaesthetic agents before or during the study. At each check-up, the patients were asked whether, despite any local pain, they wanted to stay in the study. If not, they were offered lateral internal sphincterotomy.

If the fissure persisted at the 2-month evaluation, the examiner could decide to re-treat a patient. The



Figure 1. Technique of injection.

re-treated patients received the same units of botulinum neurotoxin in the same site as the first injection. Re-treated patients were then evaluated with the same protocol 1 month and 2 months after rescue treatment. The healed patients were followed up clinically until December 2002.

Statistical analysis

All statistical analyses were performed using Statistica for Windows (Statsoft, Tulsa, OK, USA). The results were expressed as the mean \pm standard deviation; differences between manometric data were compared by Student's *t*-test for paired samples, whereas differences between percentages were analysed by Fisher's exact test. All *P* values were two-tailed; *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

One hundred and thirty-four consecutive out-patients were assessed for eligibility; of these, 24 did not meet the inclusion criteria and 10 refused to participate. One hundred patients were randomized. All patients reported severe pain after defecation, and all had a posterior anal fissure with a large sentinel tag of skin and exposure of fibres of the internal anal sphincter (Figure 2). Subjects were randomized into two groups as described above: 50 received injections of 50 units of Botox formulation (group I), and 50 received injections of 150 units of Dysport formulation (group II).

The baseline characteristics are reported in Table 1. The two groups were comparable in terms of age ($P = 0.7$), gender distribution ($P = 0.3$), duration of symptoms ($P = 0.8$) and resting pressure ($P = 0.5$) and maximum voluntary contraction ($P = 0.8$) at anorectal manometry.

The internal anal sphincter was easily palpated in all patients. No complications during the procedure or post-injection side-effects were observed in any patient. The latency of the effect was 19.1 ± 3.4 days in group I and 17.2 ± 4.1 days in group II ($P = 0.01$).

One month after the injection, inspection revealed a healing scar in 41 patients (82%) in group I and in 42 patients (84%) in group II ($P = 1.0$). Overall, symptomatic improvement was observed in nine patients (18%) in group I and in eight patients (16%) in group II. Compared with pre-treatment, post-defecation pain and bleeding disappeared in all patients.



Figure 2. Direct observation of a chronic anal fissure, with sharp borders and exposure of fibres of the internal anal sphincter.

Table 1. Baseline characteristics of 100 patients with chronic anal fissure

Characteristic	Group I (Botox formulation)	Group II (Dysport formulation)
Number of patients	50	50
Age (years)	41.6 ± 13.9	42.6 ± 14.0
Ratio of men to women	27 : 23	24 : 26
Duration of symptoms (months)	13.4 ± 10.7	13.1 ± 11.5
Symptoms (number of patients)		
Post-defecation pain	50	50
Post-defecation bleeding	31	27
Resting anal pressure (mmHg)	101.4 ± 20.8	104.1 ± 19.4
Maximum voluntary contraction (mmHg)	90.3 ± 34.0	89.2 ± 44.2

Values are mean ± s.d. All patients were included in all evaluations.

Nineteen patients (11 in group I and eight in group II, $P = 0.4$) had mild incontinence of flatus that lasted 3 weeks after treatment, but disappeared spontaneously.

At the same time (Table 2), in patients in group I (Botox formulation), the mean resting pressure and maximum voluntary squeeze pressure were 38.7% ($P = 0.00001$) and 22.4% ($P = 0.001$) lower, respec-

Table 2. Anal pressures before and after treatment with botulinum neurotoxin in patients in group I (Botox formulation)

Time	Resting anal pressure	Maximum voluntary contraction
Baseline	101.4 ± 20.8	90.3 ± 34.0
1 month	62.1 ± 15.9	70.0 ± 29.0
2 months	59.0 ± 17.0	72.0 ± 32.1

Values are mean ± s.d. Data are expressed in mmHg. All patients were included in all evaluations.

Table 3. Anal pressures before and after treatment with botulinum neurotoxin in patients in group II (Dysport formulation)

Time	Resting anal pressure	Maximum voluntary contraction
Baseline	104.1 ± 19.4	89.2 ± 44.2
1 month	61.0 ± 13.0	70.0 ± 15.0
2 months	60.0 ± 12.0	71.0 ± 30.0

Values are mean ± s.d. Data are expressed in mmHg. All patients were included in all evaluations.

tively, than the respective baseline values. In patients in group II (Table 3), the mean resting pressure was 43.1% lower than the baseline value ($P = 0.00001$), and the maximum voluntary squeeze pressure was also significantly lower (21.5% lower than the baseline value, $P = 0.004$). The anal pressure values did not differ significantly between the two groups (resting anal pressure, $P = 0.7$; maximum voluntary squeeze pressure, $P = 1.0$).

At the 2-month evaluation (Figure 3), 46 patients (92%) in group I and 47 patients (94%) in group II had a healing scar ($P = 0.7$). Symptomatic improvement was observed in four patients in group I and in three patients in group II. Compared with pre-treatment, post-defecation pain had disappeared in all patients. Post-defecation bleeding disappeared in all patients who had previously reported it.

In group I patients, the mean resting anal pressure was similar to the 1-month value ($P = 0.3$) and was 41.8% lower than the baseline value ($P = 0.00001$). The maximum voluntary squeeze pressure did not differ significantly from the 1-month value ($P = 0.7$) and was 20.2% lower than the baseline value ($P = 0.006$). In patients in group II, the resting anal pressure and maximum voluntary squeeze pressure were 60.0 ± 12.0 mmHg and 71.0 ± 30.0 mmHg, respectively. The resting pressure was 42.3% lower than the baseline



Figure 3. Chronic anal fissure healed 2 months after botulinum neurotoxin treatment.

value ($P = 0.00001$) and did not vary from the 1-month value ($P = 0.6$). The maximum voluntary pressure did not differ significantly from the 1-month value ($P = 0.8$) and was 20.4% lower than the baseline value ($P = 0.01$).

As observed at the 1-month evaluation, the anal pressure values did not differ significantly between the two groups (resting anal pressure, $P = 0.7$; maximum voluntary squeeze pressure, $P = 0.8$).

In the four patients in group I in whom the anal fissure had not healed at the 2-month evaluation, a rescue treatment was proposed. All patients refused. Inspection at 3 months after treatment revealed a healing scar in all patients. The healed patients were followed up for an average of 22.4 ± 6.0 months. During this time, no relapse occurred. No complications or side-effects were reported during follow-up in this group of patients.

At the 2-month evaluation, 47 patients in group II had healed and were not re-treated. Three patients with a persistent fissure in the absence of symptoms were re-injected with 150 Dysport units at the same site as the first treatment. Inspection at 1 month after the second injection revealed a healing scar in all patients. The healed patients were evaluated periodically. The follow-up period averaged 21.8 ± 5.1 months; during

this time, no relapse of anal fissure occurred. No complications or side-effects were reported during the follow-up period in this group of patients.

DISCUSSION

The injection of botulinum neurotoxin A, which inhibits acetylcholine release, into the internal sphincter close to an anal fissure was first described in 1994.¹³ Since then, this modality has gained popularity as a treatment for chronic anal fissure. In one study, 73% of anal fissures were healed at 8 weeks, with no recurrences at a mean of 16 months later.¹¹ Botulinum neurotoxin injection has also been compared with topical nitroglycerin ointment and, at 8 weeks, fissures were healed in 96% of patients injected with botulinum toxin and in 60% of those treated with nitroglycerin.⁴ In neither group was there recurrence at a mean follow-up of 15 months.

Previous studies have demonstrated a healing rate in the range 60–76% following a single injection of 15 or 20 units of type A botulinum neurotoxin into the internal anal sphincter.^{11, 14} Recently, we have documented that a dosage of 20 units is more effective in producing long-term healing without increasing complications.⁹ However, in all of these studies, botulinum neurotoxin was injected, close to the fissure, in the posterior aspect of the internal anal sphincter. However, when using botulinum neurotoxin for the first time at a fixed dose, variable clinical responses can be observed. These may be caused by several factors, including the dilution volume used, a single or multiple strategy of injection, the presence or absence of specific antibodies, variability in the amount of active drug present in a single vial, the susceptibility of cholinergic cells to neurotoxin, the ability of these cells to bind and internalize the toxin, and the presence of an appropriate intracellular target.

In our experience, patients with a posterior chronic fissure show better results, represented by a decrease in resting anal tone and early development of a healing scar, when type A botulinum neurotoxin is injected anteriorly into the internal anal sphincter.¹² The practice of anteriorly placed injections induces a higher fall in resting pressure and improves the clinical outcome. Fibrosis of the internal anal sphincter, which is more prominent at the site of the fissure than elsewhere in the smooth muscle,¹⁵ may reduce its compliance and limit the diffusion of botulinum neuro-

toxin. The myenteric plexus with myenteric ganglia is located between the circular and longitudinal smooth muscle layers along the entire extent of the internal anal sphincter.¹⁶ A chronic reduction of perfusion in the posterior part of the anus^{3, 17} may affect the myenteric nervous fibres at this location and make them less sensitive to the action of type A botulinum neurotoxin.

The optimal dose of botulinum neurotoxin is unclear.^{1, 2, 7} The therapeutic efficacy of different doses of botulinum neurotoxin in chronic anal fissure has been reported recently.¹⁸ It was found that the healing rate does not differ significantly when the total dose and the number of injection sites are varied. The healing rate was 83% in patients treated with 10 Botox units, 78% in those treated with 15 units and 90% in those treated with 21 units.¹⁸ Between-group comparison did not reveal significant differences. The injections were administered through the intersphincteric groove in the direction of the internal anal sphincter; however, 1 month after treatment, the mean squeeze pressure was reduced more than the resting pressure, suggesting diffusion of the toxin to the external sphincter.

In a recent study, the influence of different dosage regimens injected anteriorly into the internal anal sphincter on the clinical outcome of patients with a posterior chronic anal fissure was investigated.¹⁰ At 1 month after injection, complete healing was present in 73% of patients treated with 20 units (group I) and in 87% of patients treated with 30 units (group II). A symptomatic fissure persisted in only five patients in group I. At 2 months after injection, a healing scar was present in 89% of patients in group I and in 96% in group II; three patients in group II had a persistent fissure in the absence of symptoms. Resting anal pressures were significantly lower than pre-treatment values in both groups; although the maximum voluntary pressure was unchanged in patients treated with 20 units, it was significantly lower than the pre-treatment value in patients treated with 30 units, probably related to a diffusion of botulinum neurotoxin to the external anal sphincter.¹⁰ Five patients receiving the higher dose showed a greater reduction in sphincter tone and reported transient incontinence.¹⁰

In the current study, we investigated the efficacy and tolerability of two different formulations of type A botulinum neurotoxin to provide more evidence on the choice of dosage regimens (50 Botox units or 150 Dysport units) injected into the anterior site of the

internal anal sphincter. The results showed that, when a biological potency relationship of 3 : 1 is assumed between Dysport and Botox, the effect, efficacy and tolerability are similar. Although Dysport seemed to be more potent (the latency of the effect was 19.1 ± 3.4 days in the Botox group and 17.2 ± 4.1 days in the Dysport group; $P = 0.01$), fissure healing and symptomatic improvement were achieved in both groups of patients. The healing rate achieved for fissures in this study was comparable with that reported in most other controlled trials, and was no higher than that observed in our previous studies.^{4, 10-12, 14} However, 19 patients showed mild incontinence of flatus. These results confirm that higher doses of type A botulinum neurotoxin lead to an increase in complications and side-effects. At the 1- and 2-month evaluations, the maximum voluntary squeeze pressure was significantly lower than the pre-treatment value in both groups of patients. This was probably related to the diffusion of botulinum neurotoxin to the external sphincter.

The diffusion of botulinum neurotoxin in the tissues is a dose-dependent phenomenon. Histochemical staining of acetylcholinesterase has suggested that higher doses produce a biological effect throughout the entire muscle, whereas smaller doses produce a gradient down the length of the muscle studied.¹⁹⁻²¹ Furthermore, diffusion of the toxin outside the targeted muscles, the so-called 'toxin jump', may be responsible for the side-effects associated with the use of botulinum neurotoxin. We have noted that the decrease in anal pressure is a dose-dependent phenomenon. In previous studies, after the injection of 20 units posteriorly, the mean resting pressure, with respect to the baseline value, was 23-27% lower at the 1-month evaluation and 22-28% lower at the 2-month evaluation, whereas the maximum voluntary squeeze pressure was not changed significantly.^{9, 12} Furthermore, in recent studies, we have shown that, by choosing a different injection site (anterior aspect of the internal anal sphincter) and by using a higher dose (30 Botox units), a greater decrease can be induced in both the resting anal pressure (from 26% lower than the baseline value with posterior injection to 32% lower than the baseline value with anterior injection; and from 32% lower than the baseline value after 20 units to 35% lower than the baseline value after 30 units) and maximum voluntary contraction (15% lower than the baseline value after 30 units).^{4, 10, 12, 14}

On the basis of these results, we can conclude that botulinum neurotoxin may be considered as an

effective treatment in patients with chronic anal fissure. The efficacy and tolerability of the two different formulations of type A botulinum neurotoxin, assuming a conversion factor between their biological potencies of three, were indistinguishable. Furthermore, we do not believe that higher doses are necessary, as we were able to produce an adequate effect using lower doses. We believe that higher doses will increase the costs and contribute to the incidence of undesired effects.

REFERENCES

- 1 Utzig MJ, Kroesen AJ, Buhr HJ. Concepts in pathogenesis and treatment of chronic anal fissure — a review of the literature. *Am J Gastroenterol* 2003; 98: 968–74.
- 2 Madoff RD, Fleshman JW. AGA technical review on the diagnosis and care of patients with anal fissure. *Gastroenterology* 2003; 124: 235–45.
- 3 Lund JN, Scholefield JH. Aetiology and treatment of anal fissure. *Br J Surg* 1996; 83: 1335–44.
- 4 Brisinda G, Maria G, Bentivoglio AR, Cassetta E, Gui D, Albanese A. A comparison of injections of botulinum toxin and topical nitroglycerin ointment for the treatment of chronic anal fissure. *N Engl J Med* 1999; 341: 65–9.
- 5 Brisinda G, Maria G, Bentivoglio AR, Albanese A. The role of botulinum toxin in gastrointestinal disorders. In: Brin MF, Hallett M, Jankovic J, eds. *Scientific and Therapeutic Aspects of Botulinum Toxin*, 1st edn. Philadelphia: Lippincott Williams & Wilkins, 2002: 269–85.
- 6 Brisinda G, Maria G. Botulinum toxin in the treatment of chronic anal fissure. *Dis Colon Rectum* 2003; 46: 1144–7.
- 7 Hoogerwerf WA, Pasricha PJ. Botulinum toxin for spastic gastrointestinal disorders. *Baillieres Best Pract Res Clin Gastroenterol* 1999; 13: 131–43.
- 8 Brisinda G, Civello IM, Albanese A, Maria G. Gastrointestinal smooth muscles and sphincters spasms: treatment with botulinum neurotoxin. *Curr Med Chem* 2003; 10: 603–23.
- 9 Maria G, Brisinda G, Bentivoglio AR, Cassetta E, Gui D, Albanese A. Botulinum toxin injections in the internal anal sphincter for the treatment of chronic anal fissure: long-term results after two different dosage regimens. *Ann Surg* 1998; 228: 664–9.
- 10 Brisinda G, Maria G, Sganga G, Bentivoglio AR, Albanese A, Castagneto M. Effectiveness of higher doses of botulinum toxin to induce healing in patients with chronic anal fissures. *Surgery* 2002; 131: 179–84.
- 11 Maria G, Cassetta E, Gui D, Brisinda G, Bentivoglio AR, Albanese A. A comparison of botulinum toxin and saline for the treatment of chronic anal fissure. *N Engl J Med* 1998; 338: 217–20.
- 12 Maria G, Brisinda G, Bentivoglio AR, Cassetta E, Gui D, Albanese A. Influence of botulinum toxin site of injections on healing rate in patients with chronic anal fissure. *Am J Surg* 2000; 179: 46–50.
- 13 Gui D, Cassetta E, Anastasio G, Bentivoglio AR, Maria G, Albanese A. Botulinum toxin for chronic anal fissure. *Lancet* 1994; 344: 1127–8.
- 14 Maria G, Sganga G, Civello IM, Brisinda G. Botulinum neurotoxin and other treatments for fissure-in-ano and pelvic floor disorders. *Br J Surg* 2002; 89: 950–61.
- 15 Brown AC, Sumfest JM, Rozwadowski JV. Histopathology of the internal anal sphincter in chronic anal fissure. *Dis Colon Rectum* 1989; 32: 680–3.
- 16 Albanese A, Brisinda G, Mathias CJ. The autonomic nervous system and gastrointestinal disorders. In: Appenzeller O, ed. *The Autonomic Nervous System. Part II. Dysfunctions*. Amsterdam: Elsevier, 2001: 613–63.
- 17 Lund JN, Binch C, McGrath J, Sparrow RA, Scholefield JH. Topographical distribution of blood supply to the anal canal. *Br J Surg* 1999; 86: 496–8.
- 18 Minguez M, Melo F, Espi A, *et al.* Therapeutic effects of different doses of botulinum toxin in chronic anal fissure. *Dis Colon Rectum* 1999; 42: 1016–21.
- 19 Pearce LB, Borodic GE, Johnson EA, First ER, MacCallum R. The median paralysis unit: a more pharmacologically relevant unit of biologic activity for botulinum toxin. *Toxicon* 1995; 33: 217–27.
- 20 Simpson LL, Maksymowych AB, Hao S. The role of zinc binding in the biological activity of botulinum toxin. *J Biol Chem* 2001; 276: 27 034–41.
- 21 Simpson LL. Identification of the characteristics that underlie botulinum toxin potency: implications for designing novel drugs. *Biochimie* 2000; 82: 943–53.