Influence of Botulinum Toxin Site of Injections on Healing Rate in Patients with Chronic Anal Fissure

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BACKGROUND: Botulinum toxin induces healing in patients with idiopathic anal fissure.

METHODS: Fifty patients affected by posterior anal fissure were treated with 20 units of botulinum toxin, injected in the internal anal sphincter on each side of the posterior midline (group I) or on each side of the anterior midline (group II).

RESULTS: At 2 months evaluation, a healing scar was observed in 15 patients of group I and in 22 patients of group II ($P = 0.025$). Resting anal pressure was significantly different from the baseline values at 1-month as well as at 2-month check-ups in both groups, but the values were significantly lower in patients of group II.

CONCLUSIONS: The intersite comparison revealed that anterior injection of the internal anal sphincter resulted in improved lowering of resting anal pressure and produced an earlier healing scar.
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Anal fissure is a distressing condition. The etiology of chronic fissure remains controversial,[1] although spasm of the internal anal sphincter has been recognized to play a central role in the pathogenesis of the disease.[2–4] Surgical sphincterotomy, which is widely performed to provide symptomatic relief and healing, is highly effective[5] but can be associated with permanent complications.[2,6]

Two alternative approaches (chemical denervation with botulinum toxin and topical application of nitroglycerin ointments) have been proposed[7–10] in order to treat this condition without any risk of permanent internal anal sphincter injury. Chemical denervation with botulinum toxin is a versatile tool for targeted weakening of smooth muscle in the gastrointestinal tract.[11,12] The advantage in employing injections of botulinum toxin into the internal anal sphincter in patients suffering from chronic fissure is that the ensuing reduction in resting tone for 4 or more months should allow the fissure to heal, thus removing the need for surgery.

Previous studies[9,10] have demonstrated a healing rate ranging from 60% to 76% following a single infiltration of 15 or 20 units, respectively, of botulinum toxin in the internal anal sphincter. Recently, we have documented that a 20-unit dosage is more effective in producing long-term healing without increasing complications.[13] However, in all these studies botulinum toxin was injected, close to the fissure, in the posterior aspect of the internal anal sphincter.

We hypothesized that, at a fixed dose of 20 units, the choice of a different injection site could ameliorate the clinical outcome and could induce a higher fall of resting anal pressure. This hypothesis was based on the concept that fibrosis of the internal sphincter has been found in the base of the fissure and is more prominent in this zone than other sites in the smooth muscle.[14] The fibrosis may reduce compliance of the internal anal sphincter, limiting the toxin diffusion. Moreover, recent findings suggest that ischemia of the posterior midline may play an important part in the pathogenesis of the disease,[15–17] explaining the predilection of the fissure for this position, the lack of granulation tissue in the base of chronic fissure, and the absence of epidermal regrowth. In view of these findings a prospective, randomized study of patients with chronic fissure was initiated to provide more evidence with regard to the choice of the better site of injection of botulinum toxin in the internal anal sphincter.

PATIENTS AND METHODS

Study Patients

Consecutive symptomatic adults affected by chronic anal fissure were enrolled in the study. The diagnosis was based upon the following clinical criteria: (1) evidence of posterior circumscribed ulcer with a large sentinel tag of skin, induration at the edges and exposure of the horizontal fibers of the internal anal sphincter; and (2) symptoms (postdefecatory and/or nocturnal pain, bleeding) persistently present for over 2 months. Exclusion criteria were acute fissure, anal fissure associated with different pathologies (ie, inflammatory bowel diseases, human immunodeficiency virus infections, hemorrhoids, fistula-in-ano, anal abscesses, anal or perianal cancer) and previous surgical procedures on the anal canal.

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.

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TABLE I
Baseline Characteristics of 50 Patients with Chronic Anal Fissure

Characteristics	Group I	Group II	*P*
Age (years)	39.60 ± 13.35	45.60 ± 14.63	0.072
Ratio of men to women	14:11	11:14	0.28
Duration of symptoms (months)	16.68 ± 15.67	16.44 ± 15.62	0.95
Resting pressure (mm Hg)	108.80 ± 29.76	100.68 ± 27.22	0.32
Maximum voluntary contraction	83.00 ± 38.54	84.52 ± 31.20	0.89

Plus-minus values are means ± SD.

Study Design

This was a randomized, double-blind study. Eligible patients were randomly assigned to one of the two treatment groups according to a computer-generated list. The treating physician (GM) did not know the randomization code. Patients affected by posterior anal fissure were randomized into two groups, both receiving the injection in the internal anal sphincter: group I received the injections on each side of the posterior midline, group II received the injections on each side of the anterior midline.

At 2-month follow-up, one examiner (GB), blinded on the sites of injection, evaluated the outcome. The outcome of each group was evaluated clinically and by comparing the strength of the internal and external anal sphincters as measured by anorectal manometry. The study end point was evaluation of complete healing after botulinum toxin injections. Success was considered to be fissure healing, while persistence of the anal fissure was considered as a failure even when a symptomatic improvement occurred.

Baseline Assessment and Operative Technique

All the patients underwent a pretreatment 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.[9,10] The resting anal tone and the maximal squeeze pressure (ie, the maximal voluntary increase above the resting tone) were measured according to a stationary pull-through technique.[18] One and 2 months after the treatment, patients underwent the same evaluation performed at the baseline.

Type A botulinum toxin (Botox, Allergan, Irvine, California) was diluted in saline to 50 U/mL. The internal anal sphincter was palpated and injected with a 27-G needle, with the patient lying on the left side. Neither sedation nor local anesthesia was used during the procedure. After the baseline evaluation, 20 units of botulinum toxin were administered to patients of both groups.

Clinical Care, Follow-up, and Outcome Measures

No patient was treated with topical anesthetic agents before or during 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 retreat a patient ("rescue" treatment). The retreated patients received 25 units of botulinum toxin in the same site of the first injection. Retreated patients were then evaluated with the same protocol 1 month and 2 months after the rescue treatment. The healed patients were followed up clinically until June 1998.

Statistical Analysis

All statistical elaborations were obtained by using Statistica for Windows (Statsoft, Tulsa, Oklahoma). The results were expressed as mean ± standard deviation (SD); differences between manometric data were compared by Student's *t* test for paired samples, while differences between percentages were analyzed by Fisher's exact test.[19] *P* values of less than 0.05 were considered statistically significant.

RESULTS

From January 1996 to June 1997, 50 consecutive outpatients with chronic posterior anal fissure were enrolled. All the patients reported severe typical pain after defecating, and each had a posterior anal fissure with a large sentinel tag of skin and exposure of fibers of the internal anal sphincter. Subjects were randomized in the two groups as described above: 25 received injections of 20 units of botulinum toxin on each side of the posterior midline (group I), and 25 received injections of same amount on each side of the anterior midline (group II).

The baseline characteristics are reported in **Table I**. The internal anal sphincter was easily palpated in all patients. No complications during the procedure or postinjection side effects were observed in any patient.

One month after the injection inspection revealed a healing scar in 12 patients of group I ($P = 0.0001$ versus baseline assessment) and in 22 patients of group II ($P = 0.0001$ versus baseline, $P = 0.0027$ versus group I). At the same time, in patients of group I (posterior injection) the mean resting pressure was 22.6% lower than the baseline value ($P = 0.000005$); the maximum voluntary squeeze pressure was not significantly changed. In patients of group II (anterior injection) the mean resting pressure was 30.9% lower than the baseline value ($P = 0.000001$), and the maximum voluntary squeeze pressure was relatively unchanged **(Table II)**. Resting anal pressure was significantly lower in patients of group II when compared with patients of group I ($P = 0.022$). The values of the maximum voluntary pressure did not differ between the two groups ($P = 0.74$).

At 2-month evaluation, 15 patients (60%) of group I had a healing scar ($P = 0.00001$ versus baseline and $P = 0.57$ versus 1-month evaluation). Inspection confirmed a healing scar in the 22 patients (88%) of group II who healed

TABLE II

Anal Pressures before and after Treatment in Both Groups of Patients

	Group I		Group II	
Time	Resting Pressure	Maximum Contraction	Resting Pressure	Maximum Contraction
Baseline	108.80 ± 29.76	83.00 ± 38.54	100.68 ± 27.22	84.52 ± 31.20
1 month	84.20 ± 24.00	79.20 ± 43.02	69.52 ± 17.67	83.36 ± 39.94
2 months	84.40 ± 28.84	79.20 ± 36.98	68.56 ± 14.33	82.72 ± 38.68

Values are means ± SD. Data are expressed in mm Hg. All patients were included in all evaluations.

after 1 month ($P = 0.00001$ versus baseline evaluation, $P = 0.025$ versus group I).

In group I patients the mean resting anal pressure was similar with respect to the 1-month value ($P = 0.95$) and was 22.4% lower than the baseline value ($P = 0.00003$). Maximum voluntary squeeze pressure did not differ significantly from baseline ($P = 0.43$) and from 1 month ($P = 1.0$) values. In patients who received injections on each side of the anterior midline (group II), resting anal pressure and maximum voluntary squeeze pressure were 68.56 ± 14.33 mm Hg and 82.72 ± 38.68 mm Hg, respectively. Resting pressure was 31.9% lower than the baseline values ($P = 0.0000001$) and did not vary from 1-month values ($P = 0.74$). Maximum voluntary pressure did not differ significantly from baseline ($P = 0.76$) and from 1-month ($P = 0.84$) values.

As observed at 1-month evaluation, resting anal pressure was significantly lower in patients of group II when compared with patients of group I ($P = 0.030$), while maximum voluntary pressure remained unchanged ($P = 0.77$).

To the remaining 10 patients of group I, a rescue treatment was proposed at the 2-month evaluation. Four of them refused and underwent surgical sphincterotomy; the other 6, all of whom reported postdefecatory pain, received 25 units of botulinum toxin each in the same site of the first injection. Inspection at 1 month after rescue treatment revealed a healing scar in 5 patients. Two months after the rescue injection, complete healing was observed in the same 5 patients. The sixth patient underwent surgical sphincterotomy. The healed patients were followed up for an average 18.36 ± 7.07 months. During this time no relapse occurred in any of them. No complications or side effects were reported during follow-up of this group of patients.

At 2 months, 22 patients of group II were healed and were not retreated. Three patients were reinjected with the rescue dose of botulinum toxin (25 units) in the same site of the first treatment. Inspection at 1 month after the second injection revealed a healing scar in all patients. The healed patients were periodically evaluated. Follow-up averaged 20.08 ± 3.60 months; during this time no relapse of anal fissure occurred. No complications or side effects were reported during the follow-up period of this group of patients.

In the patients who underwent a complete treatment, fissure healing was observed in 20 out of 25 patients (80%) of group I and in all patients (25 cases) of group II ($P = 0.025$).

COMMENTS

Botulinum toxin exerts its effects on the peripheral nerve endings at the neuromuscular junction, resulting in a flaccid paralysis,[11] due to irreversible and selective multiphasic blockade of acetylcholine.[20] However, in addition to its potent skeletal neuromuscular blockade, botulinum toxin is also capable, within the autonomic nervous system, of blocking ganglionic nerve endings, postganglionic parasympathetic nerve endings, and those parasympathetic nerve endings at which acetylcholine is the transmitter.[21,22] This inhibition, first suggested in vitro,[23] was later demonstrated in vivo, in which botulinum toxin could inhibit contraction of the gastrointestinal smooth muscle.

Therefore, botulinum toxin injected into the internal anal sphincter is effective in treating anal fissure, avoiding permanent complications. In the present study, all patients were treated with the active drug and healed after one (74%) or two (90%) successive treatments. The healing rate was higher than in previous studies. In an open-label study,[9] we have observed that 6 of 10 patients healed following a single infiltration of 15 units of botulinum toxin into the internal sphincter. A success rate of 76% of the cases following a single treatment with 20 units was achieved in a double-blind study.[10] An extremely low incidence of side effects and a lack of complications were observed in a prospective comparison between two dose regimens injections (15 versus 20 units): a symptomatic improvement was achieved in both groups of patients, even if the higher dosage was more effective in inducing fissure healing.[13]

The results of the present work also confirm that the use of higher doses and the availability of a rescue treatment account for a higher success rate, without any increase of complications or side effects. As previously reported, manometric data have shown that the internal anal sphincter is weakened after the injection and that no significant diffusion to the external sphincter takes place.

However, when using botulinum toxin for the first time at fixed doses, variable clinical responses can be observed. These may be caused by several factors including the dilution volume used, a single or multiple strategy for 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 and the ability of these cells to bind and internalize the toxin, as well as the presence of an appropriate intracellular target.[20] Nevertheless, we hypothesized that botulinum toxin action on the internal anal sphincter depends upon the muscle

fiber response, as in the skeletal musculature,[24] and that the toxin is more effective in normal, nonischemic sphincter.

In the present study, we investigated the influence of different injection sites on the clinical outcome. In all patients a posterior chronic anal fissure was diagnosed and, differing from all our previous experiences[9,10,13] in which the toxin was injected in the posterior site, the injection was performed either posteriorly close to the fissure (group I) or anteriorly (group II). Injection on either side of the anterior midline was more effective in inducing fissure healing than the injection in the posterior site. Anorectal manometry showed that resting anal pressure was significantly lowered in comparison with pretreatment values in both group of patients, but the pressures were significantly lower in patients of group II when compared with patients of group I at 1-month and at 2-month evaluations; at the same time, maximum squeeze pressures did not change.

Brown and coworkers[14] have underlined that a myositis involving the internal sphincter, close to the fissure site, can develop early, causing spasm and fibrosis. The fibrosis was more prominent in the base of the fissure than laterally in the muscle, but fibrous tissue in the lateral site of the internal anal sphincter was greater than in normal subjects.[14] The fibrosis could reduce compliance of the internal sphincter and may block the action of botulinum toxin. Furthermore, the results of an angiographic postmortem study revealed that the small branches of both inferior rectal arteries passing though the internal anal sphincter have no, or only minimal, contact at the posterior commissure in 85% of cases.[15] The blood supply of the anoderm at the posterior midline was found to be significantly lower than at the other sides of the anal canal.[16] A significant relationship between anal pressure and anodermal blood flow was demonstrated at the posterior midline and morphometric study of the capillaries revealed them to be less dense both in the subanodermal space and within the internal anal sphincter. Nevertheless, the myenteric plexus with myenteric ganglia was located between the circular and longitudinal smooth muscle layers for the entire extent of the internal anal sphincter.[25,26] It seems conceivable that fibrosis or ischemia in the base of the posterior fissure may destroy the myenteric nervous fibers and, when using botulinum toxin in this site, a variable clinical response can be observed. However, independently from fibrosis, pharmacological alterations of the internal anal sphincter were found in patients affected by chronic anal fissure; a significant supersensitivity was observed in patients undergoing relaxation using isoproterenol, a selective β-adrenergic receptor agonist.[27] This phenomenon could be the results of a modification of cholinergic and adrenergic receptors or a change in their structure. Furthermore, an abnormal anorectal inhibitory reflex and a longer high-pressure zone have been noted in patients with chronic anal fissure.[28,29]

Botulinum toxin may induce healing by simply increasing local blood flow or by a more complex effect. It has been shown, in fact, that in the pig ileum botulinum toxin does not block nonadrenergic noncholinergic responses,[23] which are mediated by nitric oxide.[30,31] O'Kelly and coworkers[30] demonstrated the presence of neurones containing nitric oxide synthase in the rectal myenteric plexus that ramified throughout the internal sphincter and lay in close proximity to smooth muscle cells. The relaxation of the internal anal sphincter occurs through this subpopulation of enteric neurones containing nitric oxide.[25] Based on this evidence, it seems conceivable that botulinum toxin may bring about a local increase of nitric oxide,[32] and that this increase may be higher in the anterior injection site, in which the internal anal sphincter is normal and nonischemic and myositis is absent.

REFERENCES

1. Schouten WR, Briel JW, Auwerda JJA, et al. Anal fissure: new concepts in pathogenesis and treatment. *Scand J Gastroenterol*. 1996;31(suppl 218):78–81.
2. Lund JN, Scholefield JH. Aetiology and treatment of anal fissure. *Br J Surg*. 1996;83:1335–1344.
3. Gibbons CP, Read NW. Anal hypertonia in fissures: cause or effect? *Br J Surg*. 1986;73:443–445.
4. Farouk R, Duthie GS, MacGregor AB, et al. Sustained internal sphincter hypertonia in patients with chronic anal fissure. *Dis Colon Rectum*. 1994;37:424–429.
5. Oh C, Divino CM, Steinhagen RM. Anal fissure. *Dis Colon Rectum*. 1995;38:378–382.
6. Fazio VW. Fissure in ano. In: Keighley MRB, Pemberton JH, Fazio VW, Parc R, eds. *Atlas of Colorectal Surgery*. New York: Churchill Livingstone; 1996:105–110.
7. Simons AJ, Beart RW. Glyceryl trinitrate for anal fissure. *Lancet*. 1996;348:491–492.
8. Lund JN, Scholefield JH. A randomised, prospective, double-blind, placebo-controlled trial of glyceryl trinitrate ointment in treatment of anal fissure. *Lancet*. 1997;349:11–14.
9. Gui D, Cassetta E, Anastasio G, et al. Botulinum toxin for chronic anal fissure. *Lancet*. 1994;344:1127–1128.
10. Maria G, Cassetta E, Gui D, et al. A comparison of botulinum toxin and saline injection for treatment of chronic anal fissure. *NEJM*. 1998;338:217–220.
11. Jankovic J, Brin MF. Therapeutic uses of botulinum toxin. *NEJM*. 1991;324:1186–1194.
12. Pasricha PJ, Ravich WJ, Hendrix TR, et al. Intrasphincteric botulinum toxin for the treatment of achalasia. *NEJM*. 1995;332:774–778.
13. Maria G, Brisinda G, Bentivoglio AR, et al. Botulinum toxin injections in the internal anal sphincter for the treatment of chronic anal fissure. Long-term results after two different dose regimens. *Ann Surg*. 1998;228:664–669.
14. Brown AC, Sumfest JM, Rozwadowski JV. Histopathology of the internal anal sphincter in chronic anal fissure. *Dis Colon Rectum*. 1989;32:680–683.
15. Klosterhalfen B, Vogel P, Rixen H, Mittermayer C. Topography of the inferior rectal artery: a possible cause of chronic, primary anal fissure. *Dis Colon Rectum*. 1989;32:43–52.
16. Schouten WR, Briel JW, Auwerda JJ. Relationship between anal pressure and anodermal blood flow. The vascular pathogenesis of anal fissure. *Dis Colon Rectum*. 1994;37:664–669.
17. Schouten WR, Briel JW, Auwerda JJ, De Graaf EJ. Ischaemic nature of anal fissure. *Br J Surg*. 1996;83:63–65.
18. Cherry DA, Rothenberger DA. Pelvic floor physiology. *Surg Clin North Am*. 1988;68:1217–1230.
19. Dixon WJ, Massey FJ Jr. *Introduction to Statistical Analysis*. 4th ed. New York: McGraw-Hill; 1983.
20. Jankovic J, Hallett M. *Therapy with Botulinum Toxin*. New York: Marcel Dekker; 1994.
21. Bhutani MS. Gastrointestinal uses of botulinum toxin. *Am J Gastroenterol*. 1997;92:929–933.

22. Simpson LL. The origin, structure, and pharmacological activity of botulinum toxin. *Pharmacol Rev*. 1981;33:155–188.
23. MacKenzie I, Burnstock G, Dolly JO. The effects of purified botulinum neurotoxin type A on cholinergic, adrenergic and non-adrenergic, atropine-resistant autonomic neuromuscular transmission. *Neuroscience*. 1982;7:997–1006.
24. Borodic GE, Ferrante R, Pearce LB, et al. Histologic assessment of dose-related diffusion and muscle fiber response after therapeutic botulinum A toxin injections. *Mov Dis*. 1994;9:31–39.
25. Goyal RK, Hirano I. The enteric nervous system. *NEJM*. 1996;334:1106–1115.
26. Gershon MD, Kirchgessner AL, Wade PR. Functional anatomy of the enteric nervous system. In: Johnson LR, ed. *Physiology of the Gastrointestinal Tract*. 3rd ed. New York: Raven Press; 1994:381–422.
27. Regadas FSP, de O Batista LK, Albuquerque JLA, et al. Pharmacological study of the internal anal sphincter in patients with chronic anal fissure. *Br J Surg*. 1993;80:799–801.
28. Kuypers HC. Is there really sphincter spasm in anal fissure? *Dis Colon Rectum*. 1983;26:493–494.
29. Keck JO, Staniunas RJ, Coller JA, et al. Computer-generated profiles of the anal canal in patients with anal fissure. *Dis Colon Rectum*. 1995;38:72–79.
30. O'Kelly T, Brading A, Mortensen N. Nerve mediated relaxation of the human internal anal sphincter: the role of nitric oxide. *Gut*. 1993;34:689–693.
31. Burleigh DE. Ng-Nitro-L-Arginine reduces nonadrenergic, noncholinergic relaxations of human gut. *Gastroenterology*. 1992; 102:679–683.
32. Mariotti R, Bentivoglio M. Botulinum toxin induces nitric oxide synthase activity in motoneurons. *Neurosci Lett*. 1996;219:25–28.