Diseases of the Colon & Rectum

Gonyautoxin: New Treatment for Healing Acute and Chronic Anal Fissures

Rogelio Garrido, M.D.,[1] Nestor Lagos, Ph.D.,[2] Karinna Lattes, M.D.,[2] Mario Abedrapo, M.D.,[1] Gunther Bocic, M.D.,[1] Aldo Cuneo, M.D.,[1] Hector Chiong, M.D.,[1] Christian Jensen, M.D.,[1] Rodrigo Azolas, M.D.,[1] Ana Henriquez, T.M.,[1] Carlos Garcia, M.Sc.[2]

[1] *Departamento de Cirugia, Seccion Coloproctologia, Hospital Clinico Universidad de Chile, Santiago, Chile*
[2] *Lab. Bioquiimica de Membrana, Dept. de Fisiologia y Biofisica, Facultad de Medicina, Universidad de Chile, Santiago, Chile*

PURPOSE: The mayor symptoms of chronic anal fissure are permanent pain, intense pain during defecation that lasts for hours, blood in the stools, and sphincter cramps. It is subsequent to formation of fibrosis infiltrate that leads to an increased anal tone with poor healing tendency. This vicious circle leads to fissure recurrence and chronicity. This study was designed to show the efficacy of gonyautoxin infiltration in healing patients with anal fissures. METHODS: Gonyautoxin is a paralyzing phytotoxin produced by dinoflagellates. Fifty recruited patients received clinical examination, including proctoscopy and questionnaire to evaluate the symptoms. Anorectal manometries were performed before and after toxin injection. Doses of 100 units of gonyautoxin in a volume of 1 ml were infiltrated into both sides of the anal fissure in the internal anal sphincter. RESULTS: Total remission of acute and chronic anal fissures were achieved within 15 and 28 days respectively. Ninety-eight percent of the patients healed before 28 days with a mean time healing of 17.6 ± 9 days. Only one relapsed during 14 months of follow-up. Neither fecal incontinence nor other side effects were observed. All patients showed immediate sphincter relaxation. The maximum anal resting pressures recorded after two minutes decreased to 56.2 ± 12.5 percent of baseline. CONCLUSIONS: Gonyautoxin breaks the vicious circle of pain and spasm that leads to anal fissure. This study proposes gonyautoxin anal sphincter infiltration as safe and effective alternative therapeutic approach to conservative, surgical, and botulinum toxin therapies for anal fissures. [Key words: Anal fissure; Anal sphincters; Gonyautoxin; New treatment]

Supported by grants FONDECYT 1020090 and CSMAR 02/5-2, DID, Universidad de Chile.

Correspondence to: Nestor Lagos, Ph.D., Lab. Bioquimica de Membrana, Dept. de Fisiologia y Biofisica, Facultad de Medicina, Universidad de Chile, Casilla 70005, Correo 7, Santiago, Chile, e-mail: nlagos@med.uchile.cl

Anal fissure is a cut or crack in the anal canal that may extend from the mucocutaneous junction to the dentate line. This is a common problem that causes substantial morbidity with a roughly equal incidence in both genders and shows great reluctance to heal without intervention.[1]

Classic symptoms are pain during or after defecation that often is severe and may last for several hours. In most cases, there is bright blood on the toilet paper. The cause of chronic fissure and the reasons for the failure to heal remain unclear. Also unexplained are the main characteristics of this painful condition, including the predilection for posterior midline and the lack of granulation tissue at the fissure site.

Spasm of the internal anal sphincter has been associated with anal fissure,[2] and for many years treatment has focused on alleviating hypertonia of the sphincter. Since 1951, the common treatment for chronic anal fissure in the United States and Europe has been lateral internal sphincterotomy.[3] This surgical procedure has fundamental drawbacks, such as

permanent alterations in the control of gas, mucus, and stool.[1,4,5]

Besides the sphincterotomy, medications also have been used to temporarily create the effect of a sphincterotomy, reducing the anal tone. Two such approaches, injection of botulinum toxin[6,7] and topical application of nitroglycerin[8,9] or nifedipine ointments,[10] have been used to treat chronic anal fissure avoiding internal anal sphincter (IAS) permanent injury risk.

Microscopic planktonic algae produce phytotoxins. In the sea, these toxins are accumulated by filter feeders like bivalves.[11] When humans consume these bivalves, they become intoxicated. Until now, six human illnesses associated with phytotoxins have been described, one of them is paralytic shellfish poisoning (PSP), whose primary clinical symptom is acute paralytic illness.[12–15] This poison is formed by a mixture of phytotoxins and their high toxicity is caused by the reversibly binding to its receptor site on the voltage-gated sodium channel on excitable cells, therefore, blocking neuronal transmission. Local application of small amounts of paralyzing toxins produces a flaccid paralysis of striated muscle for periods that are dose dependant.[14–20]

Until now 26 different PSP toxins that occur naturally have been described,[11,12,21–25] all of them analogs of saxitoxin, the first one isolated[25] and the most studied one. PSP toxins are nonprotein, low molecular weight compounds and can be classified according to its net charge at neutral pH into three groups: 1) saxitoxins group net charge, +2; 2) gonyautoxins group net charge, +1; 3) group of sulfocarbamoyl toxins net charge, zero.[12]

This study was designed to examine gonyautoxin local infiltration efficacy in the treatment of anal fissure by reduction the anal tone, allowing fissure to heal and thus eliminating the need for surgery, and to propose a safe and effective alternative therapeutic approach to botulinum toxin and ointment therapies. This report describes a new therapy for anal fissure involving injection of gonyautoxin into the IAS.

PATIENTS AND METHODS

This study was performed at the Coloproctology Section, Surgery Department, Universidad de Chile Clinical Hospital, Santiago, Chile. Fifty Hispanic adults, aged 18 to 70 years, diagnosed with symptomatic anal fissures were enrolled in the study (Table 1).

Table 1.
Characteristics of Patients With Anal Fissure

Characteristic	No. of Patients (N = 50)
Male/female ratio	17/33
Mean age (yr)	35.9 ± 13.1
Spontaneous pain	46
Postdefecatory pain	50
Bleeding	49
Mean duration of symptoms (mo)	25
Acute/chronic	17/33
Localization (posterior/anterior/bilateral)	35/10/5
History of constipation	42
Previous conservative treatment	36
Previous surgical treatment	2

Data are numbers or means ± standard deviations.

Inclusion criteria: evidence of anterior, posterior, or both circumscribed ulcers, induration at the edges and exposure of horizontal fibers of the internal anal sphincter, with symptoms of postdefecatory or permanent pain, bleeding, or both. Patients with fissure history of more than two months were considered as having chronic anal fissure. Two patients suffered from chronic anal fissure for more than 21 years and five for more than 6 years with periods of intense pain and bleeding. All of them suffered from this pathology since their last childbirth. This explains the high symptoms mean duration value (Table 1).

Exclusion criteria: patients younger than aged 18 or older than 70 years, pregnancy, and fissure associated with other conditions, such as hemorrhoids, fistula, or anal abscesses. The healing criteria and primary end points of the study were pain relief and fissure epithelization. This study complied with the Declaration of Helsinki recommendation regarding biomedical research involving human volunteers and was approved by the Institutional Review Board. The design and purpose of the study and the potential risks of participation were discussed with each of the volunteers before enrollment, and their written, informed consent was obtained. This study was conducted under approval from the Universidad de Chile Clinical Hospital Ethics Committee and Public Health Institute (Reference No. 00062), Santiago, Chile.

The study was initially proposed as a randomized, double-blind trial in which the patients consecutively, as they arrived to the Coloproctology Section, were diagnosed and injected with toxin or placebo solution. After 15 patients were treated with placebo (a toxin-free 0.9 percent NaCl solution), the double-

blind trial was open for humanitarian and ethic reasons, because after three weeks the patients injected with toxin were healthy and the ones injected with placebo showed no improvement or worsening of their clinical condition. The double-blind trial was open with the authorization of Chilean Public Health Institute.

Each dose of toxin tested consisted in a sterile solution of 100 units of gonyautoxins 2/3[11,26] in 1 ml total volume of 0.9 percent of sodium chloride, without preservatives. This dose was locally infiltrated in both sides of the fissure, in the IAS using 0.5 ml in each side. An insulin syringe with 25-gauge needle (25 × 5) was used for the injection. One unit of the paralyzing toxin activity corresponds to the amount of toxins enough to block neuromuscular contraction of mouse leg crural bicep for 1.5 to 2 hours. The gonyautoxins were purified from shellfish highly contaminated with PSP toxins. The shellfish were collected in Chilean Patagonia fjords.[11,26]

Anal pressures were measured by recording resting and voluntary contraction pressures at baseline. Two minutes after injection, a second anorectal manometry was performed, also testing the integrity of both the anorectal inhibitory and anocortical reflexes of each participant before and after the toxin injection.

Manometric recordings and an analysis of the tracings were made using a water-perfusion system. The anal canal pressure was recorded by stationary pull-through technique using a water-filled microballoon and external transducer (PVB) perfusion equipment (Medtronic Inc., Bonn, Germany). The recording and analysis of the tracing were both made by a computerized system (8 channels polygraph ID, Medtronic Polygraph with Polygram 98 version 2.2 software). Anal resting pressures were recorded in millimeters of mercury using the stationary pull-through technique and the computer identified the mean pressure. The maximal voluntary contraction was assessed by evaluation of the voluntary contractions of anal sphincter in each participant. Amplitude was expressed in millimeters of mercury.

The patients were clinically evaluated after 7, 14, 21, and 28 days from the day of injection. The injection pain scores and the pain two minutes after the injections were evaluated by asking patients to rate their pain in a Visual Analog Scale from 1 to 10, with 10 being the maximum pain value. The patients did not receive stool softeners, bulk laxatives, or sitz baths during the study. Long-term outcomes were determined after 14 months follow-up.

Table 2.
Symptoms and Side Effects in Patients After Treatment

	No. of Patients
No. of doses needed	2.6 ± 1.3
Mean healing time (days)	17.6 ± 9
Pain during injection (maximum, 10; minimum, 1)	5.8 ± 2.3
Pain two minutes after injection (maximum, 10; minimum, 1)	1.6 ± 1.2
Duration of bleeding after first injection (days)	1.2 ± 0.9
Duration of pain after first injection (days)	2.1 ± 0.7
Flatus incontinence	None
Fecal incontinence	None
Digital examination (sphincter immediate relaxation)	100
Side effects	None

Data are means ± standard deviations or percentages.

RESULTS

No patients dropped out of the study or were lost to the follow-up monitoring during the study, and none suffered adverse events or negative side effects during or after this study. One hundred percent of patients showed immediate postinjection anal sphincter relaxation (Table 2). This relaxation was detected by digital examination and anorectal manometry. The patients declared that they felt anal anesthesia after injection. During the digital examination, coloproctologists detected the anal tone reduction, which persisted in the seven-day postinjection examination. No incontinence was observed (Table 2). One hundred percent of the patients maintained the integrity of the anorectal inhibitory and anocortical reflexes (Table 3).

Manometric recordings showed significant decrease in maximum resting pressure (MRP) in all patients injected. MRP after two minutes was 61.1 ± 22.8 mmHg (mean value ± standard deviation), decreasing to 56.2 ± 12.5 percent of baseline values, representing a mean reduction of 43.8 percent. Similarly, maximum voluntary contraction pressure (MVCP) decreased to 74.3 ± 13.1 percent of baseline, representing a mean reduction of 25.7 percent (Table 3).

All patients with acute anal fissures stopped bleeding within 48 hours after injection. At the first clinical control (7 days), they showed epithelization of the lesion with modest pain only after defecation. At the second clinical examination (14 days), they were totally epithelized with scar formation. During this control, digital examination was possible with normal minor discomfort; all of them healed completely within 15 days.

Table 3.
Anorectal Manometry Recordings

	No. of Patients (N = 50)
MRP	
Before injection (mmHg)	108.8 ± 24.9
Two minutes after injection (mmHg)	61.1 ± 22.8
% of MRP, two minutes after injection	56.2 ± 12.5
MVCP	
Before injection (mmHg)	159.9 ± 32.8
Two minutes after injection (mmHg)	118.8 ± 12.7
% of MVCP, two minutes after injection	74.3 ± 13.1
Reflexes	
Recto-anal reflex (% maintained)	100
Cortical-anal reflex (% maintained)	100

MRP = maximum resting pressure; MVCP = maximum voluntary contraction pressure.

Data are means ± standard deviations or percentages.

Chronic anal fissure patients also stopped bleeding within 48 hours, but only 50 percent of the patients showed epithelization during the first clinical control. Nevertheless, the epithelization was evident in the second control (14 days) and was complete 14 days later in 98 percent of patients. By this time (28 days), all of them were free of pain and asymptomatic. One patient (59-year-old female) relapsed after three months and required surgical intervention. She had an eight-year history of recurrent anal fissures, with periods of intense pain and bleeding, complicated with subfissural infiltration and bleeding tendency. Her anorectal manometry at baseline showed an MRP of 118 mmHg and MCVP of 171 mmHg. Up to this moment, and after 14 months of follow-up, the other 49 patients enrolled in this trial were asymptomatic and healthy.

DISCUSSION

The fact that 100 percent of the patients immediately relaxed the anal sphincter after infiltration shows that this toxin, locally injected, produces paresis in the internal sphincters and reduces the anal tone. Important findings in this study are that neither gas nor fecal incontinence was observed and that all patients maintained their anorectal inhibitory and the anocortical reflexes completely functional, suggesting that the infiltration blocks extra contraction of the muscle but leaves enough strength for normal performance. Local dose reinjections were performed without problems or side effects.

The gonyautoxin toxicity is caused by the reversibly binding to its receptor site on the voltage-gated sodium channel on excitable cells, thus blocking the influx of Na^+ ions and preventing nerve and muscle cells from producing action potentials, therefore, blocking neuronal transmission, which results in a temporary paralysis of muscles.[16,17] Gonyautoxin paralyzes the injected sphincter reducing anal tone, leaving the other muscles unaffected. The paretic effect lasts for more than one week. No side effects were observed in the patients during a 14-month follow-up, which points to the safety of this therapeutic approach.

Of 50 patients, 3 acute and 33 chronic (36 in total) were treated before this trial with conservative treatments, such as sitz bath, stool softeners, high-fiber diet, bowel regulation, and topical ointments. None of them healed with those treatments.

The healing rate of both acute and chronic anal fissures obtained in this trial was 98 percent within 28 days (49 patients); however, in the acute anal fissure group, 100 percent of 17 patients were healed within 15 days. These results showed better efficacy compared with other pharmacologic treatments, such as botulinum toxin injection,[7,27,28] nitroglycerin,[8,9,29] or nifedipine ointment applications.[10]

Chronic anal fissures treated with nitroglycerin showed a healing rate ranging from 36 to 60 percent within two to six months.[10,29–32] The most recent report showed an inexplicably high placebo response with 50 percent of healing.[32] Treatment with nitroglycerin manifests, as side effect, moderate-to-severe headaches.[10,30–32] The application of this ointment during three months causes a decrease in the MRP ranging from 17 to 38 percent without significant change in MVCP.[1,8,10,30,31]

Topical nifedipine treatment also has been used for healing chronic anal fissure, with a healing rate ranging from 50 to 94.5 percent within six weeks.[33,34] This topical application does not produce negative side effects, reducing MRP in only 11 percent of the baseline without alteration in MVCP.[34] Recurrence is frequent in both ointment treatments: 31 percent of patients with nitroglycerin and 42 percent of those treated with nifedipine.[10]

Botulinum toxin has been used as treatment for idiopathic anal fissure since 1993,[27] with healing rates

ranging from 60 to 96 percent within two to six months.[7,27,28,31,35–37] The healing rate is associated with the regimen dosage, which allowed healing in 96 percent of the cases in long-term treatments (6–12 months) using two doses of toxin.[37,38] This toxin reduced anal tone in all patients from the fifth day after injection.[27] It is well known that botulinum toxin begins producing its relaxant effect in approximately five days in all the expanding spectrum of its clinical uses. After two months of botulinum toxin treatment, the MRP was reduced in a 28.4 percent, without significant change in the MVCP.[31] The treatment with this toxin produces a chemical denervation that lasts more than three months and exhibits approximately 10 percent of temporal fecal incontinence with 20 percent recurrence.[28,38] No other side effects have been reported.[7]

The tissue immobilization is a healing fundamental therapeutic principle. The treatment with both toxins originates chemical denervation, which produces muscle paralysis when injected locally. The question that arises is why produce a three-month or more chemical denervation when only 20 days are required to heal anal fissures?

The therapy developed here answers this question. Because of the gonyautoxin physiologic effect, blocking the voltage-gated sodium channel in a total reversibly way,[18,19,39] its effect is dose-dependent and the binding to its receptor site is only regulated by affinity. Therefore, any mass equilibrium change in the extracellular body fluid, close to the infiltrated area (diffusion gradient), will favor the toxin dissociation with the following reduction of the paretic effect. The effect of the 100-unit dose used in this trial lasted for 12 days. In contrast, botulinum toxin produced an irreversible chemical denervation that lasted more than three months with the consequent proteolytic structural damage. Muscle inactivation persists until new fibrils grow from the nerve and form junction plates on new areas of the muscle-cell walls.[40]

The immediate sphincter relaxation produced by gonyautoxin and the volume injection in which the toxin is delivered (0.5 ml each side) are crucial for the rate and time of anal fissure healing. Both are distinctive features of these toxins compared with the other pharmacologic treatments. The injected volume is important because, even when the toxin is infiltrated into the internal anal sphincter, because of its small thickness, 1-ml volume of toxin should spread to the proximity and reach the external anal sphincter. This explains the impressive fall of MRP and MVCP measured in this study.

CONCLUSIONS

Because of the outstanding effectiveness and safety shown by gonyautoxin in this trial, gonyautoxin local infiltration into IAS, on an outpatient basis, produced a temporary pharmacologic immobilization of the anal sphincter muscles that eliminated sphincter spasm-the critical step that breaks the vicious circle of damage, pain, and sphincter spasm, thus healing anal fissures. Gonyautoxin infiltration represents a new therapeutic approach for acute and chronic anal fissures and because of its efficacy it should be preferable to other pharmacologic treatments and surgery. The latter has the inconveniences of permanent fecal incontinence risk, five to seven weeks of healing time, sphincter damage, and costly hospitalization.

REFERENCES

1. Lund JN, Scholefield JH. Aetiology and treatment off anal fissure. Br J Surg 1996;83:1335–44.
2. Farouk R, Duthie GS, MacGregor AB, Bartolo DC. Sustained internal anal sphincter hypertonia in patients with chronic anal fissure. Dis Colon Rectum 1994;37: 424–9.
3. Eisenhammer S. Surgical correction of chronic internal anal (sphincteric) contracture. S Afr Med J 1951;25: 486–9.
4. Khubchandani IT, Reed JE. Sequelae of internal sphincterotomy for chronic fissure in ano. Br J Surg 1989;76: 431–4.
5. Hsu TC, MacKeigan JM. Surgical treatment of chronic anal fissure: a retrospective study of 1753 cases. Dis Colon Rectum 1984;27:475–8.
6. Jost WH, Schimrigk K. Therapy of anal fissure using botulin toxin. Dis Colon Rectum 1994;37:1321–4.
7. Jost WH. One hundred cases of anal fissure treated with botulin toxin: early and long-term results. Dis Colon Rectum 1997;40:1029–32.
8. Loder PB, Kamm MA, Nicholls RJ, Phillips RK. Reversible chemical sphincterotomy by local application of glyceryl trinitrate. Br J Surg 1994;81:1386–9.
9. Gorfine SR. Topical nitroglycerin therapy for anal fissures and ulcers. N Engl J Med 1995;333:1156–7.
10. Ezri T, Susmallian S. Topical nifedipine vs. topical glyceryl trinitrate for treatment of chronic anal fissure. Dis Colon Rectum 2001;46:805–8.
11. Lagos N. Microalgal bloom: a global issue with negative impact in Chile. Biol Res 1998;31:375–86.
12. Oshima Y. Postcolumn derivatization liquid chromato-

graphic method for paralytic shellfish toxins. J AOAC Int 1995;78:528–32.

13. Andrinolo D, Michea LF, Lagos N. Toxic effects, pharmacokinetics and clearance of Saxitoxin, a component of paralytic shellfish poison (PSP), in cats. Toxicon 1999;37:447–64.
14. Lagos N, Andrinolo D. Paralytic shellfish poisoning (PSP): toxicology and kinetics. In: Botana LM, ed. Seafood and freshwater toxins: mode of action, pharmacology and physiology. New York: Marcel Dekker I, 2000:203–15.
15. Andrinolo D, Iglesias V, Garcia C, Lagos N. Toxicokinetics and toxicodynamics of gonyautoxins after an oral toxin dose in cats. Toxicon 2002;40:699–709.
16. Kao CY. Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomenon. Pharm Rev 1966;18:997–1049.
17. Narahashi T. Mechanism of action of tetrodotoxin and saxitoxin on excitable membranes. Fed Proc 1972;31: 1124–32.
18. Catterall WA, Morrow CS, Hartshorne RP. Neurotoxin binding to receptor sites associated with voltage-sensitive sodium channels in intact, lysed, and detergent-solubilized brain membranes. J Biol Chem 1979; 254:11379–87.
19. Moczydlowski E, Hall S, Garber SS, Strichartz GS, Miller C. Voltage-dependent blockade of muscle Na+ channels by guanidinium toxins: effect of toxin charge. J Gen Physiol 1984;84:687–704.
20. Strichartz GS, Hall S, Magnani B, Hong CY, Kishi Y, Debin JA. The potencies of synthetic analogues of saxitoxin and the absolute stereoselectivity of decarbamoyl saxitoxin. Toxicon 1995;33:723–37.
21. Harada T, Oshima Y, Yasumoto T. Structure of two paralytic shellfish toxins. Gonyautoxins V and VI, isolated from a tropical dinoflagellate, Pyrodinium bahamense var. compressa. Agric Biol Chem 1982;46: 1861–4.
22. Onodera H, Satake M, Oshima Y, Yasumoto T, Carmichael WW. New saxitoxin analogues from the freshwater filamentous cyanobacterium Lyngbya wollei. Nat Toxins 1997;5:146–51.
23. Lagos N, Onodera H, Zagatto PA, Andrinolo D, Azevedo SM, Oshima Y. The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium Cylindrospermopsis raciborskii, isolated from Brazil. Toxicon 1999;37:1359–73.
24. Molica R, Onodera H, Garcia C, *et al*. Toxins in the freshwater cyanobacterium Cylindrospermopsis raciborskii, isolated from Tabocas reservoir in Caruaru, Pernambuco, Brazil. Phycologia 2002;41:606–11.
25. Schantz EJ, Ghazarossian VE, Schones HK, Strong FM. The structure of saxitoxin [letter]. J Am Chem Soc 1975; 97:1238–9.
26. Lagos N. Paralytic shellfish poisoning phytotoxins: occurrence in South America. Comments Toxicology 2003;9:1–19.
27. Jost WH, Schimrigk K. Use of botulinum toxin in anal fissure [letter]. Dis Colon Rectum 1993;36:974.
28. Gui D, Cassetta E, Anastasio G, Bentivoglio AR, Maria G, Albanese A. Botulinum toxin for chronic anal fissure. Lancet 1994;344:1127–28.
29. Lund JN, Scholefield JH. A randomized, prospective, double-blind, placebo-controlled trial of glyceryl trinitrate ointment in treatment of anal fissure. Lancet 1997; 349:11–4.
30. Bacher H, Mischinger H-J, Werkgartner G, *et al*. Local nitroglycerin for treatment of anal fissures: an alternative to lateral sphincterotomy ? Dis Colon Rectum 1997; 40:840–5.
31. 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.
32. Bailey HR, Beck DE, Billingham RP, *et al*. A study to determine the nitroglycerin ointment dose and dosing interval that best promote the healing of chronic anal fissures. Dis Colon Rectum 2002;45:1192–9.
33. Antropoli C, Perrotti P, Rubino M, *et al*. Nifedipine for local use in conservative treatment of anal fissures: preliminary results of a multicenter study. Dis Colon Rectum 1999;42:1011–5.
34. Perrotti P, Bove A, Antropoli C, *et al*. Topical nifedipine with lidocaine ointment vs active control treatment of chronic anal fissure: results of a prospective, randomized, double-blind study. Dis Colon Rectum 2002;45: 1468–75.
35. Jost WH, Schimrigk K. Botulinum toxin in therapy of anal fissure. Lancet 1995;345:188–9.
36. Maria G, Casetta E, Gui D, Brisinda G, Bentivoglio AR, Albanese A. A comparison of botulinum toxin and saline for treatment of chronic anal fissure. N Engl J Med 1998;338:217–20.
37. Maria G, Brisinda G, Bentivoglio AR, Casetta 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.
38. 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.
39. Kao CY, Nishiyama A. Action of saxitoxin on peripheral neuromuscular systems. J Physiol 1965;180:50–66.
40. Borodic GE, Pearce LB. New concepts in Botulinum toxin therapy. Drug Saf 1994;11:145–52.

Invited Commentary

To the Editor—Not long ago, a proposal to inject a highly lethal, biologic toxin to treat a benign condition would have been thought absurd. Such thinking became obsolete with the advent of Botox® (Allergan, Irvine, CA), and botulinum toxin therapy for disorders as various as spastic torticollis, blepharospasm, anal fissure, and aesthetically unpleasing facial wrinkles has now gained widespread acceptance. Accordingly, we should not be surprised to learn that a different biologic toxin, gonyautoxin, is now being proposed as a novel treatment for anal fissure, or that it appears to be efficacious.

Despite steady improvement in study design and increasing emphasis on randomized, controlled trials, the optimal treatment of chronic anal fissure remains uncertain. For years, lateral internal sphincterotomy (LIS) was standard therapy for medically refractory fissures, although reports of high rates of minor incontinence dampened enthusiasm for the procedure.[1] Meanwhile, "chemical sphincterotomy" using topical agents such as glyceryl trinitrate (GTN) and diltiazem, and injectable agents such as botulinum toxin, rapidly gained popularity as early reports suggested excellent results without the incontinence risk of LIS. A cottage industry of identifying and studying agents pharmacologically related to those known to relax the internal anal sphincter was born, and the search was on for newer and better nitrogen donors and calcium channel blockers. Meanwhile, new reports emphasizing noncompliance because of adverse effects (particularly headache from GTN)[2] and high recurrence rates[3,4] began to appear, and one randomized, controlled trial of GTN *vs.* LIS suggested significant advantages to surgery in the first instance.[5] The current disarray in the literature is exemplified by two very recent and virtually simultaneous publications: a review by Lindsey and associates, which concluded that "first-line use of medical therapy cures most chronic anal fissures cheaply and conveniently, . . ."[6] and a systematic review by Nelson, which concluded that —". . . medical therapy for chronic anal fissure may be applied with a chance of cure that is only marginally better than placebo . . . [and] far less effective than surgery."[7]

In the present study by Garrido *et al.* reported a 98 percent fissure-healing rate after injection of 100 units of gonyautoxin into the internal anal sphincter. The study group comprised patients with both acute and chronic anal fissures. Although initiated as a randomized, controlled trial, the study design was changed to a single-treatment arm after preliminary analysis showed a large disparity in results in the treatment and control groups. The authors demonstrate almost immediate (within 2 minutes) relaxation of the internal sphincter in 100 percent of patients. Side effects, including fecal and or gas incontinence, were not observed in any patient during 14-month follow-up. These results are impressive—indeed, dramatic—and as such they raise the obvious question: are they too good to be true? Experience from other nonoperative therapies for fissure suggests that initial reports of success do not guarantee similar results from other centers, or similar long-term success. It is encouraging to read that gonyautoxin is a more effective sphincter relaxant than other available agents, and perhaps this is the reason for its high level of efficacy, but one must wonder if its relatively short duration of action might translate eventually into more relapses.

A bigger issue relates to the use of gonyautoxin itself. Paralytic shellfish toxins such as gonyautoxin are elaborated planktonic algae, accumulate in shellfish and cause human disease when the affected shellfish are eaten. They work by blockading sodium channels, which leads to loss of neuronal transmission. There is some literature on human paralytic shellfish poisoning, usually in the form of small case series, but exceedingly little on the toxin itself. Its use as a therapeutic agent has not previously been reported. Accordingly, a number of fundamental questions must be answered before gonyautoxin therapy can be widely advocated. Is there a standardized method of toxin acquisition and purification? What is a safe dose? What is the optimal dose? What is the toxicity, and what are the associated risks? How do we treat an overdose? In the United States, the Food and Drug Administration will properly need to see proof of safety and efficacy before it approves the sale of gonyautoxin. The question then will be: how much does it cost?

Garrido and colleagues deserve tremendous credit for describing a truly novel treatment for anal fissure. Although the authors are to be applauded for ingenuity and outstanding results, readers should remain aware of the numerous questions that await answers, particularly regarding gonyautoxin pharmacology, safety, long-term outcomes, and costs. Readers also should remember that we have had high hopes for other fissure treatments in the past, only to learn of suboptimal outcomes and unsuspected side effects. As a result, we must insist on further inquiry with

appropriately designed trials before embracing this new technique.

REFERENCES

1. Garcia-Aguilar J, Belmonte C, Wong WD, Lowry AC, Madoff RD. Open vs. closed sphincterotomy for chronic anal fissure: long-term results. Dis Colon Rectum 1996; 39:440–3.
2. Altomare DF, Rinaldi M, Milito G, *et al*. Glyceryl trinitrate for chronic anal fissure-healing or headache? Results of a multicenter, randomized, placebo-controlled, double-blind trial. Dis Colon Rectum 2000;43:174–9.
3. Carapeti EA, Kamm MA, McDonald PJ, Chadwick SJ, Melville D, Phillips RK. Randomised controlled trial shows that glyceryl trinitrate heals anal fissures, higher doses are not more effective, and there is a high recurrence rate. Gut 1999;44:727–30.
4. Minguez M, Herreros B, Espi A, *et al*. Long-term follow-up (42 months) of chronic anal fissure after healing with botulinum toxin. Gastroenterology 2002;123:112–7.
5. Richard CS, Gregoire R, Plewes EA, *et al*. Internal sphincterotomy is superior to topical nitroglycerin in the treatment of chronic anal fissure: results of a randomized, controlled trial by the Canadian Colorectal Surgical Trials Group. Dis Colon Rectum 2000;43:1048–57.
6. Lindsey I, Jones OM, Cunningham C, Mortensen NJ. Chronic anal fissure. Br J Surg 2004;91:270–9.
7. Nelson R. A systematic review of medical therapy for anal fissure. Dis Colon Rectum 2004;47:422–31.

Sharon L. Dykes, M.D.
Robert Madoff, M.D.
Minneapolis, Minnesota

The Authors Reply

To the Editor—Our clinical trial to date has reached 153 patients who have been treated for anal fissure; most are chronic. Our original study was only 50 patients and has been maintained practically without change. Side effects, including fecal and/or gas incontinence, were not observed in any patient through a 24-month follow-up. In addition, we have tested gonyautoxins in more than 500 patients with the same dose (100 units) and doses reaching 10 to 20 times higher in different applications, such as blepharospasm, permanent hemifacial spasm, poststress hemifacial spasm, posthemorrhoidectomy treatment to reduce postoperative pain, hemorrhoidal flexion, torticollis, and aesthetically unpleasing facial wrinkles (all ongoing studies). In all of these cases, there have been no side effects and the outstanding effectiveness and safety observed in the anal fissure clinical trial, and these have been shown in all the clinical applications tested to date.

In regard to your question: "Is there a standardized method of toxin acquisition and purification?" There are standardized methods for toxin acquisition and purification. We have published the purity of these toxins and these epimers by HPLC analysis with fluorescent detection and mass spectroscopy review articles.[1–9]

In regards to your question: "What is the optimal dose?" The physiologic effect of this toxin is dose-dependent. This act is by the high affinity, making a reversible blocking of the sodium channel voltage-dependent. The optimal dose for each application is currently under consideration in ongoing studies and will be addressed in additional studies. We have made a formal presentation or (NDA equivalent) to our national Instituto de Salud Publica in Chile, equivalent to the American FDA.

According to our clinical experience, we conclude that for acute anal fissure only one dose of 100 units could be needed for healing. In this study, we used two doses (each one every 7 days) to follow the protocol approved and proposed by us to the ethical committee of our hospital. In this case, maybe in the future, another trial with only one dose of 200 units would be tested to develop a treatment for anal fissure with one dose that is more concentrated. At this moment, the two doses of 100 units every seven days is the proposed treatment for healing acute and chronic anal fissures according to the results of the clinical trail presented in this article.

Related to your questions regarding toxicology, pharmacology, and transport, we have developed studies and published articles associated to these topics in mammalians (cat *in vivo* experimental model, human tissue, and postmortem analysis).[10–17] Most were published before our clinical trials and were the basis of our initial proposal to the ethical committee at the Clinical Hospital, University of Chile, and our Chilean Public Health Institute.

REFERENCES

1. Compagnon D, Lembeye G, Marcos N, Ruiz-Tagle N, Lagos N. Bioaccumulation of PSP toxins in bivalve Aulacomya ater and two carnivorous gastropods Concholepas concholepas and Argobuccinum ranelliformes

during Alexandrium catenella bloom in the southern Chile. J Shellfish Res 1998;17:67–74.
2. Lagos N, Compagnon D, Andrinolo D, Salas K. Quantitative analysis of PSP toxicity in the fjord system of the southern regions of Chile: HPLC technology. Proc II Reuniao Ibero-Americana de Ficologia 1998;I:51-62..
3. Lagos N. Microalgal bloom: a global issue with negative impact in Chile. Biol Res 1998;31:375–86.
4. Lagos N, Onodera H, Zagatto PA, Andrinolo D, Azevedo SM, Oshima Y. The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium Cylindrospermopsis raciborskii, isolated from Brazil. Toxicon 1999;37:1359–73.
5. Andrinolo D, Santinelli N, Otano S, Sastre V, Lagos N. Paralytic shellfish toxins in mussels and Alexandrium tamarense at Valdes Peninsula, Chubut, Patagonia Argentina: kinetic of a natural depuration. J Shellfish Res 1999;18:203–9.
6. Pereira P, Onodera H, Andrinolo D, *et al*. Paralytic shellfish toxins in the freshwater cyanobacteria Aphanizomenon flos-aquae, isolated from Montargil reservoir, Portugal. Toxicon 2000;38:1689–702.
7. Lagos N. Principales toxinas de origen fitoplantonico: identificacion y cuantificacion mediante cromatografia liquida de alta resolucion (HPLC). In: Sar EA, Ferrario y Beatriz Reguera ME, eds. Floraciones algales nocivas en el Cono Sur Americano. Madrid: Instituto Espanol Oceanografico, 2002:55–76.
8. Molica R, Onodera H, Garcia C, *et al*. Toxins in the freshwater cyanobacterium Cylindrospermopsis raciborskii, isolated from Tabocas reservoir in Caruaru, Pernambuco, Brazil. Phycologia 2002;41:606–11.
9. Lagos N. Paralytic shellfish poisoning phytotoxins: occurrence in South America. Comments Toxicol 2003;9: 175–93.
10. Andrinolo D, Michea LF, Lagos N. Toxic effects, pharmacokinetics and clearance of Saxitoxin, a component of paralytic shellfish poison (PSP), in cats. Toxicon 1999;37:447–64.
11. Lagos N, Andrinolo D. Paralytic shellfish poisoning (PSP): toxicology and kinetics. In: Botana LM, ed. Seafood and freshwater toxins: mode of action, pharmacology and physiology. New York: Marcel Dekker, 2000:203–15.
12. Andrinolo D, Iglesias V, Garcia C, Lagos N. Toxicokinetics and toxicodynamics of gonyautoxins after an oral toxin dose in cats. Toxicon 2002;40:699–709.
13. Andrinolo D, Gomes P, Fraga S, Soares-Da-Silva P, Lagos N. Transport of the organic cations gonyautoxin 2/3 epimers, a Paralytic Shellfish Poison toxin, through the human and rat intestinal epithelium. Toxicon 2002;40: 1389–97.
14. Garcia C, Bravo MC, Lagos M, Lagos N. Paralytic shellfish poisoning: post-mortem analysis of tissue and body fluid samples from human victims in the Patagonia fjords. Toxicon 2004;43:149–58.
15. Mardones P, Andrinolo D, Csendes A, Lagos N. Permeability of human jejunal segments to gonyautoxins measured by the Ussing chamber. Toxicon 2004;44:521–8.
16. Garrido R, Lagos N, Lattes K, García C., Azolas R., Bocio G., Cuneo A., Chiong H., Jensen C., Henriquez A., Fernández C., *et al*. The gonyautoxin 2/3 epimers reduces anal tone when injected in the anal sphincter of healthy adults. Biol Research 2004;37:395–403.
17. Lagos N, Garcia C, Lattes K, *et al*. Paralytic shellfish poison: toxins that can kill and heal. Proceedings of the Chemical and Biological Medical Treatment Symposium Switzerland: Spiez Laboratory (in press).

Rogelio Garrido, M.D.
Nestor Lagos, Ph.D.
Karinna Lattes, M.D.
Mario Abedrapo, M.D.
Gunther Bocic, M.D.
Aldo Cuneo, M.D.
Hector Chiong, M.D.
Christian Jensen, M.D.
Rodrigo Azolas, M.D.
Ana Henriquez, T.M.
Carlos Garcia, M.Sc.
Santiago, Chile