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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Yo, JIMENA ESCOBAR URIBE, identificada como aparece al pie de mi firma, domiciliada en Bogotá, en la Carrera 11 A No. 94-76 Of. 305, obrando como apoderada de la sociedad BioteCon Gesellschaft für Biotechnologische Entwicklung und Consulting mbH., domiciliada en Potsdam, Alemania, solicitante de la patente de la referencia, pido a usted que se proceda a examinar si la invención, cuya patente se tramita en el expediente de la referencia, es patentable.

Adjunto al presente memorial el recibo No. 02 - 42,145 de junio 26, 2002, por la suma de \$318.000.00, con lo cual queda cubierta la tasa oficial correspondiente a dicho estudio.

Señor Superintendente,


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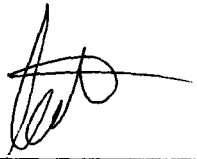
***** CONSIGNACION *****

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***** CONCEPTO *****

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US005846929A

United States Patent [19]**Johnson et al.**[11] **Patent Number:** **5,846,929**[45] **Date of Patent:** **Dec. 8, 1998**[54] **PURIFICATION OF TYPE G BOTULINUM NEUROTOXIN AND PHARMACEUTICAL COMPOSITIONS THEREOF**[75] Inventors: **Eric A. Johnson; Hiroshi Sugiyama; Carl J. Malizio**, all of Madison, Wis.[73] Assignee: **Wisconsin Alumni Research Foundation**, Madison, Wis.[21] Appl. No.: **633,092**[22] Filed: **Apr. 16, 1996****Related U.S. Application Data**

[63] Continuation-in-part of Ser. No. 287,350, Aug. 8, 1994, abandoned.

[51] Int. Cl.⁶ **A61K 38/00**, A23J 1/00; C12P 21/04[52] U.S. Cl. **514/2**; 514/21; 530/350; 530/412; 530/413; 530/416; 530/417; 530/418; 530/825; 435/68.1; 435/70.1; 435/70.3; 435/842

[58] Field of Search 514/2, 21; 530/350, 530/825, 412, 413, 416, 418, 417; 435/68.1, 70.1, 70.3, 842

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[57] **ABSTRACT**

The isolation and purification of type G botulinum neurotoxin and complexes thereof is disclosed. Compositions containing the type G neurotoxin and the preparation of a type G toxoid are also disclosed.

4 Claims, No Drawings

PURIFICATION OF TYPE G BOTULINUM NEUROTOXIN AND PHARMACEUTICAL COMPOSITIONS THEREOF

RELATED CASES

The present application is a continuation-in-part of our earlier U.S. patent application Ser. No. 08/287,350 filed Aug. 8, 1994, now abandoned.

FIELD OF THE INVENTION

The present invention relates to botulinum toxin. More particularly, it relates to a method of purifying type G botulinum neurotoxin and novel pharmaceutical compositions containing type G botulinum neurotoxin.

BACKGROUND OF THE INVENTION

The most serious form of bacterial food poisoning is botulism which is caused by neurotoxins produced by *Clostridium botulinum*. The toxins are absorbed from the intestinal tract and transported via the circulatory system to motor nerve synapses where their action blocks normal neural transmissions. There are seven serotypes of the botulinum toxin with similar toxic activity but which differ antigenically. They are type A, B, C, D, E, F and G. Type A is the predominant toxin in cases of botulism in the United States, and type B toxin is most prevalent in Europe. The symptoms for the disease caused by the various serotypes are about the same, but the mechanism of action is different. It is possible to protect humans by a pentavalent immunization against types A to E, but because of unavailability of the purified toxin, specific antitoxins are not available for type G.

Crystalline botulinum toxin type A prepared in 1979 by E. J. Schantz of the Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, Wis., has been used medicinally in the treatment of hyperactive muscle movement disorders, such as strabismus and other involuntary muscle disorders. Treatment involves injection of nanogram quantities (1 ng is equal to 30 mouse 50% lethal doses [30 U]) of the toxin directly into the hyperactive muscles. The toxin inhibits the release of acetylcholine from the motor neuron across the synaptic junction, causing a decrease in the hyperactivity of the injected muscles.

Botulinum toxin type A has become one of the most effective treatments for various types of dystonias, or involuntary muscle contractions, for many thousands of people. A major drawback to the use of botulinum toxin type A in the treatment of hyperactive muscle disorders in the development of antibodies to the type A toxin resulting in ineffective treatment with the toxin, particularly for patients treated for spasmodic torticollis which requires larger and sometimes more frequent doses of the toxin by injection. The type A toxin is recognized by patient's immune systems as a foreign protein which stimulates antibody production. The fact that antibodies to the type A toxin have been formed in patients has been verified by the U.S. Center for Disease Control and other medical units.

Although the commercially available type A preparation is very useful, there is a need to have a pharmaceutical composition of a botulinum toxin of alternative serotype which can be used in patients who have developed antibodies to the type A toxin, and the many individuals who have antibodies to types A-E, including military soldiers and researchers who have been immunized with pentavalent toxoid.

There also is a need to have a purified type G botulinum neurotoxin which can be used to prepare a toxoid to protect against type G botulism as might occur naturally or in biological warfare. Nukina et al (12,13) have attempted to obtain a purified type G toxin; however, their products are highly unstable.

BRIEF SUMMARY OF THE INVENTION

It is an object of the present invention to disclose novel pharmaceutical compositions that contain type G botulinum neurotoxin and its protein complexes and which can be used in patients who have developed antibodies to the type A toxin.

It is a further object to disclose a novel method of purifying the type G neurotoxin from a natural source so that it can be used to prepare such pharmaceutical compositions and a toxoid that can be used to immunize patients against the type G botulism.

We have discovered a method of purifying type G botulinum neurotoxin from natural sources to obtain type G neurotoxin of high purity which can be used to prepare a toxoid or for medicinal use. Since type G botulinum neurotoxin has a different mechanism of action than type A it could be used in patients who have developed antibodies to the type A toxin or in individuals immunized with A-E pentavalent toxoid.

The novel method of purifying the type G botulinum neurotoxin basically comprises growing *C. botulinum* type G under anaerobic conditions in a liquid medium culture containing trypticase-peptone and free of L-cysteine HCl precipitating the neurotoxin by adding yeast RNA and lowering the pH to about 3.4 with acid; extracting the toxin from the precipitate with a phosphate-NaCl buffer (pH 7); centrifuging the mixture and dissolving the precipitate in phosphate buffer (pH 6.0); adding RNAase and incubating the mixture at 37° C., to salt out the toxin as a precipitate; suspending the precipitated toxin in sodium citrate buffer (pH 5.5) and loading it on a DEAE-Sephadex A-50 gel; collecting the A₂₆₀/A₂₇₈ fractions with a ratio of 0.6 or less; salting out the toxin; loading it on a SP-Sephadex column; eluting the neurotoxin-containing fractions and then purifying the neurotoxin by PAPTG affinity chromatography to obtain a stable, highly purified type G neurotoxin molecule weighing 144,000 daltons. The type G toxin can be activated by treatment with a protease such as Endoproteinase Lys-C. Purified, activated type G toxin has a specific activity of greater than 1×10⁷ LD₅₀/mg protein and is greater than 90% pure by SDS-PAGE.

The novel pharmaceutical compositions of the present invention are lyophilized products which contain about 10⁵ to 5×10⁷ mouse LD₅₀'s (U) per mg of protein of active botulinum neurotoxin type G.

At the time of use, the compositions can be diluted to the desired concentration by aseptically adding physiological saline or buffers to obtain a solution suitable for injection. The desired amount of the solution (e.g. 0.1 ml) is then injected using a suitable needle, such as an electromyographic needle, into the proper or hyperactive muscle.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The preferred embodiment of the compositions of the present invention is a lyophilized product containing type G botulinum neurotoxin or its protein complex containing 10⁵ to 5×10⁷ U of activity dependent upon the degree of puri-

lication. The lyophilized product may be prepared from a prelyophilization formulation containing organic or inorganic buffers of appropriate pH and ionic strength. The toxin can also be stabilized by the addition of exogenous proteins (e.g. serum albumin) and other materials (e.g. trehalose).

The preferred method of preparing the compositions of the present invention comprises dissolving the purified botulinum type G toxin with a solubilizing amount of sodium phosphate buffer or other appropriate buffer (pH 6.2-7.4) and ultrafiltering the solution to maintain sterility. It is then dried by lyophilization for dispensing to physicians.

Type G botulinum complexes and purified neurotoxin of the present invention are produced under anaerobic conditions in liquid medium culture. The neurotoxin component has a molecular weight of 140-150 kda as determined by SDS-PAGE. Its activity is increased by treatment with Endoproteinase Lys-C. The type G toxin produced from *Clostridium botulinum* type G has a specific LD₅₀ per mg. toxicity in mice of $>1 \times 10^7$ U/mg, an A₂₆₀/A₂₇₈ ratio of less than 0.60, and a distinct pattern of banding on gel electrophoresis. The neurotoxin also can be isolated as complexes of 300-500 kDa (10^5 to 10^7 U/mg) which are more stable than the purified neurotoxin and may have even greater utility in medicine.

For lyophilization, aliquots (usually 0.1 ml) of the toxin solutions can be pipetted into 0.5 dram (ca. 1.85-ml), screw-cap glass vials with rubber-lined closures. The solutions in glass vials may then be frozen in a bath of liquid nitrogen at -200° C. or by placing them in a freezer at -20° C. or -70° C. The loosely capped vials can then be placed into a 150 ml lyophilization flask that had been precooled in liquid nitrogen. The lyophilization flask is partially immersed in liquid nitrogen and connected to a lyophilizer (Virtis Freezemobile 12, Virtis Co., Inc. Gardiner, N.Y.). The liquid nitrogen is maintained in contact with the lyophilization flask until the pressure drops to approximately 30 millitons (ca. 4.0 kPa). The liquid nitrogen jacket is then removed, the flask and contents are allowed to come to room temperature (ca. 3 to 4 h), and the drying procedure is continued for 12 to 18 h. The pressure is maintained at or below 20 millitons for the remainder of the cycle. Condenser temperature on the lyophilizer is kept constant at ca. -60° C. After the drying cycle is completed, the lyophilized toxin vials are removed from the flask, tightly capped, and held at room temperature for up to 3 days until the mouse assay. The lyophilized preparations can be reconstituted in 1.0 ml of distilled water or 0.85% saline as a diluent.

MATERIALS AND METHODS

I. Toxin Complex Production

C. botulinum Type G strain 89 (available from CDC, Atlanta Ga.) was initially obtained from a soil sample from Mendoza, Argentina. Long term storage is in cooked meat medium (Difco). Working stocks were stored in a medium (TPGY) containing 5% trypticase-peptone (BBL), 0.5% Bacto-peptone (Difco), 2% yeast extract (Difco), 0.4% glucose (Mallinckrodt), and 0.2% L-cysteine HCl (Sigma) adjusted to pH 7.3. An 8% final concentration of sterile glycerol was added to 16 to 40 hour cultures as a cryoprotectant. Cultures were stored at -60° to -80° C.

The toxin production medium was free of L-cysteine HCl and contained 2.0% trypticase-peptone (BBL), 1.0% proteose-peptone (Difco), 0.5% yeast extract (Difco), and 0.5% glucose (Mallinckrodt) adjusted to pH 7.3 (2). The cysteine HCl was omitted because we discovered that it caused cell clumping and reduced the yield of toxin. When large volumes were used, a 14% glucose solution was

autoclaved for 20 minutes and added to the large volume which was separately autoclaved for 1 hour.

After thawing, a working stock culture was mixed and 0.1-0.2 ml inoculated into a 10 ml tube of TPGY and incubated under anaerobic conditions at 30° C. After 16-40 hours incubation, 10 ml of TPGY were inoculated into 100 ml of toxin production medium. After 16 hour incubation, 100 ml were inoculated into 1400 ml of toxin production medium. Finally after 16 hours incubation, 1400 ml were inoculated into 14 liters of toxin production medium in 20 liter carboys. The carboys were incubated 6 days at 30° C. To maintain anaerobic conditions a 10% CO₂+90% N₂ gas mixture filtered through a 0.2 um filter (Gilman) was bubbled through a gas dispersion tube into the toxin production medium contained in a 20 liter carboy for 3-4 hours prior to inoculation and during the entire 6 day incubation. Exhaust from carboy was bubbled through gas dispersion tubes into two side arm flasks connected in series containing 5.25% hypochlorite. (This method gave toxin titers in the culture fluid of $>3.0 \times 10^4$ LD₅₀/ml compared to the 1.3×10^4 LD₅₀/ml obtained by Nukina et al (12), who did not use anaerobic conditions, used a different strain, and used a cysteine containing culture medium.)

Toxin purification

All buffers except acetate were made by titrations of equimolar solutions of basic and acidic components. Acetate buffer was made by addition of NaOH solution to the desired molarity of acetic acid. All chromatography was done at room temperature.

The toxin in the culture fluid was precipitated by adding 0.2 g/liter RNA (Sigma) and lowering the pH to 3.4 with 3M HCl or sulfuric acid. The addition of RNA prior to acidification made it possible to recover all of the toxin from the culture fluid. (Nukina et al (12) used 4N sulfuric acid and no RNA.) The acidified culture fluid was stored 16-72 hours at 4° C.

The toxin was extracted from the precipitate with 0.2M phosphate +0.5M NaCl buffer, pH 7.0 for two hours at room temperature. The extract was centrifuged and the clarified supernatant fluid saved as the first extract. The pellet was reextracted in the same buffer for 1 hour at room temperature and then centrifuged. The clarified supernatant fluid obtained was combined with the first extract fluid and made to 60% saturation with (NH₄)₂SO₄ (39 g /100 ml) before being stored at 4° C. (Nukina et al (12) used a different extraction buffer which lacked sodium chloride and they treated their precipitate by sonication to release toxin.) The salted out precipitate of the extraction was collected by centrifugation and dissolved in 100 ml of 0.05M phosphate buffer pH 6.0. The toxin solution was incubated at 37° C. for three hours after adding 0.05 mg/ml RNase (Sigma type 1A). This step removes the RNA associated with the toxin complex. The toxin solution was then made with 60% (NH₄)₂SO₄ (39 g /100 ml) and stored at 4° C. (In contrast, in the Nukina et al procedure, the acid precipitated extract and residual extract are then trypsinized which we have discovered results in a less stable neurotoxin molecule. Instead of trypsin, we use Endo-proteinase Lys-C at a later step in our process because it acts only at the lysine residue.)

DEAE-Sephadex chromatography. The salted out precipitate of RNase treated material was collected by centrifugation, suspended in 40 ml of 0.05M sodium citrate buffer, pH 5.5. The toxin sample was loaded on a DEAE-Sephadex A-50 gel (Sigma) that had been equilibrated and washed with citrate buffer. The toxin did not bind under these conditions and the toxin was washed out of the column with citrate buffer. The toxin came off in the first large

Use of Botulinum Toxin Type F Injections to Treat Torticollis in Patients with Immunity to Botulinum Toxin Type A

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Summary: Fifteen patients with torticollis who had been treated with repeated injections of botulinum toxin type A (botox A) developed antibodies to the toxin. This resulted in loss of benefit in the 13 patients who had improved with botox A injections and failure to develop muscle atrophy after injection in all 15 patients. Patients were then injected with botulinum toxin type F (botox F) in the same muscles that had been injected with botox A. Ten of the 15 improved after botox F injections, including 9 of the 12 patients who had improved with type A toxin. Six of 9 patients with pain had improvement in pain after botox F injections. Patients reported similar improvement with type F and type A toxins, but duration of benefit was ~3 months with type A and ~1 month with type F. Botox F is an effective treatment for torticollis in patients who are immune to botox A. The usefulness of type F toxin, however, is limited by short duration of benefit. **Key Words:** Torticollis—Dystonia—Antibody—Botulinum toxin type A—Botulinum toxin type F.

Botulinum toxin (botox) injections are now recognized as a major means of treating some forms of focal dystonia, e.g., blepharospasm (1,2), torticollis (3-9), and spastic dysphonia (10). Botox has also been used with success in treating patients with dystonia of jaw and other lower facial muscles (11), brachial dystonia (12,13), and a variety of other hyperkinetic movement disorders. Although local side effects, such as ptosis after blepharospasm injections and dysphagia after neck or tongue injections, may complicate treatment in a minority of patients, systemic side effects after therapeutic botox injections are uncommon.

In most centers, early experience with the use of botox for blepharospasm, in doses of 25-100 U every 3 months, indicated that tachyphylaxis did not occur (1 U is the LD₅₀ of the toxin in mice). Since

the regular use of much higher doses of botox for torticollis (150-300 U every 3 months), patients have now been recognized with antibodies to botox A in sufficient titers to block all clinical effect (14,15). The risk factors for development of antibodies to botox are not known, nor has there been an estimate of the prevalence of this problem in patients receiving botox therapy for torticollis. Nonetheless, immunity to botox is a major therapeutic problem for some patients.

There are seven known antigenically distinct botulinum toxins, all acting at presynaptic sites to block cholinergic neuromuscular transmission. Antibodies to type A toxoid (inactivated type A toxin) do not seem to have significant neutralizing effect against type F toxin (16), suggesting that type F toxin (botox F) might be an alternative therapy for patients who have developed antibodies to type A toxin (botox A). Botox F is less lethal in animals than botox A per unit protein (17). When injected i.m. in rats, type F causes less muscle weakness

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and has a shorter duration of action than an equivalent LD₅₀ of type A (18). The first use of botox F in patients with antibodies to botox A was reported in 2 patients with torticollis, 1 patient with oromandibular dystonia, and 1 patient with stuttering (19). We here present another series of patients treated with botox F after developing immunity to botox A.

METHODS

Since 1984, over 500 patients with torticollis have been injected with botox A at the Dystonia Clinical Research Center at Columbia-Presbyterian Medical Center. For the first injection series, most patients received a moderate dose of botox A and returned in 2 to 3 weeks for supplemental injections to maximize improvement. Some patients continued to have supplemental injection series after subsequent treatments, although in the last 2 to 3 years, we have tried to reduce this practice. For purposes of analysis, we considered two injection series that occurred within 3 weeks of each other as a single "treatment" with the combined dose of toxin. We usually required that patients wait ≥ 3 months before having repeat treatments. Patients with clinical evidence suggesting antibodies to botox A (resistance to therapy and failure to develop muscle atrophy after injection) had serum assayed by mouse bioassay in the laboratory of Dr. Charles Hatheway at the Centers for Disease Control in Atlanta, Georgia, or in the laboratory of Dr. Alan Scott at Smith-Kettlewell Eye Institute in San Francisco, California. Fifteen patients with resistance to therapy and serological evidence of antibodies to botox A agreed to participate in a trial of botox F injections. The protocol for the use of botox F was approved by the Institutional Review Board of Columbia-Presbyterian Medical Center, and all patients signed a consent form before treatment.

We received vials of botox F prepared at the Osaka Prefecture Agricultural College through the courtesy of Professor Genji Sakaguchi. The toxin was purified and tested at the Chiba Serum Institute, with an estimated 2405 U/vial. We diluted botox F in sterile normal saline without preservative. The LD₅₀ was assayed at our center in 18- to 20-g male Balb/C mice. Four mice were injected intraperitoneally with 1 of 5 serial dilutions (20 mice total). Eight mice were injected with botox A as a control. Based on the number of mice surviving over 5 days, LD₅₀ was calculated according to the method of Reed and Muench (20). Our assay estimated the strength of the toxin at 2520 U/vial. Each

vial was diluted with 50 ml normal saline without preservative to a concentration of ~ 5 U/0.1 ml. Patients were injected in the same muscles that had been injected with botox A (usually the sternocleidomastoid contralateral to rotation, and the trapezius, splenius capitus, and levator scapulae ipsilateral to tilt or rotation; patients with retrocollis were injected in both trapezeii and splenii). Because we were concerned that frequent injections or high doses of botox F might induce antibodies to botox F, most patients received a total of 250 U of botox F, independent of the dose of botox A that had been used (mean dose of botox F: 234.7 U; range: 50–350 U). The doses of botox A received by these patients ranged from 20 to 760 U (mean 277.3 U).

RESULTS

Development of Antibodies to Botox A

The 15 patients consisted of 11 women and 4 men, a proportion not significantly different from the distribution of 341 women and 218 men in all patients injected for torticollis (Chi-square). Compared to torticollis patients without known antibodies, patients with antibodies developed torticollis at a younger age (28.5 ± 17 years compared to 39.5 ± 15 years, $p < .003$ by t-test) and had a longer duration of symptoms when first injected with botox A (16.5 ± 9 years compared to 10.8 ± 12 years, $p < .03$ by t-test).

All 15 patients initially had muscle atrophy after botox A injections, but had no muscle atrophy (usually gauged after injection of 60 to 80 U into one sternocleidomastoid muscle) at the time of their last injection with botox A. Serum was obtained for antibody assay during the course of botox A injections in 8 patients. In 7 patients, serum was obtained for assay from 3 weeks to 1 year after their last botox A injection (mean delay 4.4 months, median delay 2 months). Twelve patients initially received benefit from botox A, but lost all benefit at the time that botox A injections stopped producing muscle atrophy; 3 patients never had clear-cut improvement with botox A and received multiple series of injections in an attempt to produce improvement.

The mean time between initial injections of botox A and documentation of seropositivity was 20.4 months (range 6–46 months, mean 6.5 treatments); the mean dose/treatment was 277.3 U, and the mean cumulative dose at the time of seropositivity was 1793.1 U. Because treatments were usually ~ 3 months apart, there could have been a delay of ≤ 3

months between the development of antibodies and the time of the next treatment when immunity became clinically evident. In some patients, loss of response was gradual or was not appreciated immediately. Those patients may have received several treatments before antibodies were documented. In 8 patients, there was a sharp decline in response after a series of successful injections, so that high titers of antibodies presumably developed after the last successful treatment. These 8 patients had a mean of 4.5 treatments (range: 2-6 treatments over a mean of 11.9 months (range: 3-19 months) before beginning to lose benefit; the mean dose/treatment was 310.8 U, and the mean cumulative dose before loss of efficacy was 1398.8 U. The longest time before loss of efficacy was 19 months (6 treatments); the earliest that response failure appeared was after 2 treatments (3 months).

This group of 15 patients does not represent all patients with antibodies at our Center. Several patients with serological evidence of antibodies have been lost to follow-up. Other patients have had absence of atrophy of muscles after repeated injection series, but have not had serological studies, or have had negative serological studies. Some of these patients probably also have antibodies. The initial injections in these patients were performed between 1985 and 1990. The frequency of injection sessions, concentration of toxin, number of injections per session, and total dose of toxin were different in 1990 than in 1985. For these reasons, we have not attempted to identify possible risk factors for developing immunity in this group of patients.

Response to Botox F

Patients were injected with botox F from 2 months to 5 years after their last injection with botox A (mean delay 14.7 months, median delay 7 months). Ten of the 15 patients reported subjective improvement in dystonia after injections of botox F, including 9/12 (75%) patients who initially responded to botox A (Table 1). The 9 patients who improved with both serotypes estimated the degree of improvement with botox F as similar to, or slightly less than, the degree of improvement achieved with botox A. Of 13 patients examined 2 weeks after botox F injections, 11 had marked atrophy of the sternocleidomastoid muscle after injection of 25 to 50 U of botox F. One patient refused to allow injection of the sternocleidomastoid, and 1 patient did not return for examination.

TABLE 1. Response to botox F

	Improved with botox A	No response to botox A
Improved with botox F	9	1
No response to botox F	3	2

Botox, botulinum toxin.

Mean dose of botox F, 234.7 U (range 50-350 U); mean number of treatments, 1.9 (range 1-5); duration of benefit from botox F, 4.7 weeks (3-8 weeks); longest duration of treatment with botox F, 22 mo.

Six of 9 patients with pain had resolution, or marked improvement, in pain after botox F injection, compared to improvement in pain in 7 of 9 patients after botox A injections. One of the patients whose pain improved after botox F injection had not improved after botox A injections. This patient had been one of the first patients injected for torticollis at Columbia and had received 60 to 280 U of botox A at 2- to 4-week intervals in gradually increasing doses. She never reported benefit with botox A and had detectable serum antibodies after 1 year of treatment (11 treatment sessions). She stopped developing muscle atrophy after botox injection after 3 months of treatment (6 sessions), and thus it is difficult to interpret her lack of benefit from botox A.

There was marked muscle atrophy in 2 patients who did not have clinical improvement in dystonia after botox F injection (1 had improved with botox A).

In 1 patient who had subjective improvement after botox A injections, there was no detectable atrophy of the sternocleidomastoid after 50 U of botox F, and this patient did not report improvement. There was no detectable change in pre- and post-botox F videotapes in this patient. Another patient with initial botox A improvement reported complete atrophy of the sternocleidomastoid 3 days after injection with botox F, but noted that muscle bulk had rapidly returned. At 2 weeks, the sternocleidomastoid was still atrophied to ~50% of its original bulk, and the muscle remained soft after maximal effort. This patient reported improvement in dystonia lasting only 3 to 7 days, showed no change in videotapes taken before and 2 weeks after botox F injections, and was considered a botox F failure. As indicated above, 2 of 3 patients considered botox A failures were also botox F failures. One patient who had improved with botox A and had muscle atrophy with botox F did not experience improvement in dystonia after botox F injections,

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and no change could be detected in videotapes taken before and after botox F injections.

Duration of benefit was estimated from time of injection with botox F to the time that symptoms had substantially returned. Patients were not reexamined when they reported loss of benefit, but this was comparable to the way duration of benefit was established with botox A. Most patients reported loss of benefit after 4 weeks (range 3–8 weeks) with mean 4.7 weeks. Two patients reported local pain lasting ≤ 10 days. Three patients reported mild "flu-like" syndrome—muscle aches, malaise, diarrhea, dry mouth, and sore throat, lasting ≤ 2 weeks. This flu-like syndrome occurs in $\sim 8\%$ of all our torticollis patients injected with botox A. There were no reports of excess neck weakness, which occurs in $\sim 16\%$ of injected torticollis patients at our center.

DISCUSSION

Naturally occurring antibodies to botox A have rarely been detected, even in survivors of botulism (21). Some torticollis patients treated with repeated injections of botox A develop sufficient titers of antibodies to completely block response to further injections. In at least some of these patients, antibodies can be demonstrated by mouse bioassay. The majority of our patients are seen 2 weeks to 1 month after their initial botox injection. At that time, failure to develop muscle atrophy after adequate doses has not been noted. All patients with antibodies detected by mouse bioassay failed to develop muscle atrophy after injections of high doses of botox A. Unfortunately, the mouse assay is difficult to perform and relatively insensitive. Several laboratories have used the ELISA (enzyme-linked immunosorbent assay) to measure antibotulinum toxin antibodies (22). This technique is easier to perform and standardize, but the ELISAs described to date have been less sensitive than the bioassay (22).

Some patients who fail to develop atrophy after botox A injection do not have antibodies by mouse assay. We do not know whether the assay is not sensitive enough to detect antibodies or whether absence of atrophy is explained by some other mechanism in addition to the presence of antibodies.

In our patients, there was a delay ranging between 2 months and 5 years between the time botox A injections were stopped and botox F injections were performed. It is not known how long antibodies to botox A persist. The antibody response to inoculation with botulinum toxoid has been studied

using the mouse neutralization assay (23). The antibody response to a mixture of botox A and B may decrease between 2 months and 1 year, but high titers are generally present after a booster at 1 year, and these levels persist ≥ 2 years. The antibody response to botox A alone was maintained for ≥ 5 years after a booster at 5 to 7 months (23). All of our patients received ≥ 1 injection series after they had developed antibodies so that, in effect, they all received boosters.

We have demonstrated that botox F can be used to treat torticollis in patients with antibodies to botox A. The physiological effect per unit of each serotype, gauged by degree of atrophy of an injected sternocleidomastoid muscle, is comparable. Although we have not measured the duration of the physiological effect, the brief duration of clinical benefit makes it unlikely that botox F will prove an adequate therapy for many patients with torticollis. We do not know how botulinum toxins are inactivated; the clinical effect may last longer in patients with involvement of smaller muscles, such as in blepharospasm, laryngeal dystonia, and so forth. Also, botox F may be adequate for some clinical situations in which long duration of effect is not necessary. Occasional patients with severe torticollis and radiculopathy/myelopathy need botulinum toxin injections to relax muscle spasm in the post-laminectomy period. Botox F may be preferable to botox A in this situation. High doses of botox F can be used because excess neck weakness will be of short duration. Development of immunity to botox F may not preclude future treatment of torticollis with botox A. Similarly, botox F may have a role in patients with severe, fixed dystonia of the upper or lower extremities who are considering operation to permanently weaken muscles. Botox F could be injected preoperatively to verify that weakness of the target muscles will result in functional improvement.

We only had 3 patients who responded to botox A but not to botox F. In two of these patients, absent or short-lived atrophy after botox F injection suggests that antibodies to botox A may have interfered with the action of botox F. This possibility should be resolved once sensitive assays are available for antibodies to both serotypes of toxin. So far, after almost 1 year of use, there has been no indication of immunity to botox F. The amount of protein per unit of biological activity is higher for F than for A, so that the risk of triggering antibodies to botox F remains a concern.

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Doctora
JIMENA ESCOBAR URIBE
(Apoderado)

ASUNTO	Radicación	00042309
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	8808
	Folios	014

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

Documentos estudiados:

Los textos estudiados fueron la descripción (Folios: 3 a 21) y las reivindicaciones 1 a 10 (Folios: 22 a 24).

La presente solicitud reivindica prioridad alemana N° DE19925739.6 de 07 de junio de 1999 y cumple con lo establecido en los artículos 9, 10 y 11 de la Decisión 486 de la Comisión de la Comunidad Andina.

Objeto de invención:

La presente solicitud se titula: "Preparación farmacéutica que contiene una de las neurotoxinas botulínicas de *Clostridium botulinum* de los tipos A, B, C, D, E, F, o G, o una mezcla de dos o más de estas neurotoxinas".

El objeto de la presente solicitud se refiere a preparaciones farmacéuticas que contienen una neurotoxina de *Clostridium botulinum*, donde la neurotoxina está libre de las proteínas complejizantes que están presentes de manera natural en el complejo. La consecuencia de ello es el reconocimiento en el cual se basa la presente solicitud, en el sentido de que la neurotoxina libre, contrariamente a lo que sucede con el complejo, no produce o produce en forma marcadamente disminuida, la inducción de anticuerpos neutralizadores en el paciente. La presente solicitud se refiere además al empleo de neurotoxinas botulínicas de *Clostridium botulinum* para la preparación de un medicamento para el tratamiento de enfermedades del sistema nervioso. Otro aspecto se refiere al empleo de la toxina botulínica de *Clostridium botulinum* para el tratamiento cosmético.

Según la descripción, el complejo de toxinas de *Clostridium botulinum* del tipo A es empleado desde hace varios años para el tratamiento de diversas distonías. Actualmente están autorizados dos preparados distintos que contienen este complejo, para el tratamiento del blefarospasmo (espasmo de los párpados), de los espasmos hemifaciales y del espasmo rotatorio (Torticollis spasmodicus): BOTOX y

DYSPOORT. La terapia de otras enfermedades del sistema nervioso (espasticidades, migraña, lumbalgia, síndrome cervical, hipersalivación) está siendo examinada clínicamente en la actualidad. Los preparados se emplean además en indicaciones cosméticas tales como la hiperhidrosis y la formación pronunciada de arrugas. Hasta el momento, no existe en el mercado producto alguno autorizado, conteniendo toxina del tipo B-G.

Los complejos de toxina botulínica se componen de una mezcla de proteínas clostridiales. Estas son hemaglutininas con masas moleculares diferentes, una proteína no tóxica no hemaglutinante y una neurotoxina estable frente a ácidos que es responsable de la toxicidad oral en intoxicaciones por alimentos. Contrariamente a lo que ocurre con la neurotoxina pura, el complejo resiste el medio ambiente agresivo del tracto gastrointestinal y posibilita la resorción enteral de la neurotoxina, la que alcanza las células de destino a través del torrente sanguíneo o del sistema linfático y desencadena allí un bloqueo de la liberación de transmisores. Se llega así a la parálisis de la musculatura estriada y lisa y a la extinción de diversas funciones vegetativas. Para la aplicación terapéutica, según el estado actual de la técnica, el complejo se inyecta directamente en los músculos distónicos o espásticos, donde la neurotoxina, a un pH fisiológico, es liberada del complejo y provoca la acción farmacológica deseada. A pesar de que el complejo es aplicado solo en dosis sumamente reducidas después de inyecciones reiteradas, se llega en un número considerable de pacientes a la formación de anticuerpos específicos neutralizadores que también están dirigidos contra la neurotoxina.

De acuerdo con la presente solicitud, los inventores de esta misma se propusieron la tarea de desarrollar un tratamiento alternativo para las enfermedades y trastornos antes mencionados. Los inventores quisieron, en particular, proponer una sustancia activa apropiada con la cual pueden ser tratados pacientes, que ya han desarrollado anticuerpos neutralizadores. Como solución a la tarea propuesta, se desarrolló un nuevo medicamento que contiene solo neurotoxina pura (tipo A, B, C, D, E, F, G) y que está libre de hemaglutininas y otras proteínas ajenas al cuerpo.

Lo que se reivindica como novedoso es lo siguiente: preparación farmacéutica que contiene una de las neurotoxinas botulínicas de *Clostridium botulinum* de los tipos A, B, C, D, E, F, G o una mezcla de dos o más de estas neurotoxinas, caracterizada porque la neurotoxina o bien la mezcla de neurotoxinas está libre de proteínas complejizantes que de manera natural forman los complejos de neurotoxina botulínica junto con las neurotoxinas.

El objeto de la solicitud definido anteriormente se clasificó en la IPC A61K 35/74.

Claridad de la solicitud:

El artículo 30 de la Decisión 486 de la Comisión de la Comunidad Andina establece que: "Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción."

Las reivindicaciones podrán ser independientes o dependientes. Una reivindicación será independiente cuando defina la materia que se desea proteger sin referencia a otra reivindicación anterior. Una reivindicación será dependiente cuando defina la materia que se desea proteger refiriéndose a una reivindicación anterior. Una reivindicación que se refiera a dos o más reivindicaciones anteriores se considerará una reivindicación dependiente múltiple."

De acuerdo con lo anterior, la reivindicación 1 (folio: 22) que se refiere a una preparación farmacéutica que contiene neurotoxina botulínica, no está claramente definida por su contenido o sus elementos constitutivos, sino por lo que no está

presente en la misma; además no se dice cuáles son sus excipientes que la hagan diferente a lo ya conocido en el estado de la técnica.

La reivindicación 2 (folio: 22) que se refiere a una preparación farmacéutica, no está caracterizada como tal sino por una respuesta farmacológica dada por un paciente.

Reivindicaciones de uso:

El artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina establece: "Los Países Miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial".

De acuerdo con lo anterior, la(s) reivindicación (es) 6 a 10 (Folios: 23 y 24), que se refiere(n) a el empleo de la neurotoxina botulínica de Clostridium botulinum, no se encuentra(n) dentro del campo de la patentabilidad nombrado en el artículo 14.

Determinación del estado de la técnica:

El artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina establece que "El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de Patente o, en su caso, de la prioridad reconocida.

Sólo para el efecto de la determinación de la novedad, también se considerará dentro del estado de la técnica, el contenido de una solicitud de patente en trámite ante la oficina nacional competente, cuya fecha de presentación o de prioridad fuese anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, siempre que dicho contenido esté incluido en la solicitud de fecha anterior cuando ella se publique o hubiese transcurrido el plazo previsto en el artículo 40".

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 07/06/1999, se buscaron documentos del estado de la técnica relacionados, con fecha de publicación anterior a esta.

Se hizo la búsqueda de documentos relevantes en el estado de la técnica, entendiendo como estado de la técnica la definición provista en el artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina y las excepciones fijadas en el artículo 17 de la misma.

Se encontró como anterioridades (documentos cuya fecha de publicación es anterior a la fecha de presentación de la primera solicitud de patente sobre la cual se reivindica prioridad o de la fecha de presentación de esta solicitud) que pueden afectar la patentabilidad del objeto de la presente solicitud:

Nº	Documento	Título	Fecha de Publicación
1	US 5846929	Purification of type G botulinum neurotoxin and pharmaceutical compositions thereof	8 de diciembre de 1998
2	Artículo 1	Use of botulinum toxin type F injections to treat torticollis in patients with immunity to botulinum toxin type A ¹	Octubre de 1993

1 GREENE P., FAHN S. "Use of botulinum toxin type F injections to treat torticollis in patients with immunity to botulinum toxin type A". Dystonia Clinical Research Center. New York (USA); oct. 1993.

Evaluación de los requisitos de Patentabilidad:

El Artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina el cual establece: "Los Países Miembros otorgarán Patentes para las invenciones, sean de producto o de procedimiento en todos los campos de la tecnología siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial".

Se procedió a evaluar los requisitos de novedad, nivel inventivo y aplicación industrial con el fin de establecer la patentabilidad de la invención reivindicada en la solicitud en estudio.

Evaluación de Novedad:

El artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina establece que "Una invención se considerará nueva cuando no está comprendida en el estado de la técnica". "El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida.

Sólo para el efecto de la determinación de la novedad, también se considerará dentro del estado de la técnica, el contenido de una solicitud de patente en trámite ante la oficina nacional competente, cuya fecha de presentación o de prioridad fuese anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, siempre que dicho contenido esté incluido en la solicitud de fecha anterior cuando ella se publique o hubiese transcurrido el plazo previsto en el artículo 40".

Esta se evaluó por comparación entre lo solicitado y los documentos encontrados en el estado de la técnica y que se relacionan con el objeto reivindicado y de acuerdo con la definición de novedad del Artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina.

Comparando elemento por elemento el objeto de esta solicitud con lo mencionado en las anterioridades, tenemos que:

La Solicitud de Patente Colombiana No. 00042309, se refiere a:

Preparación farmacéutica que contiene una de las neurotoxinas botulínicas de *Clostridium botulinum* de los tipos A, B, C, D, E, F o G, o una mezcla de dos o más de estas neurotoxinas, caracterizada porque la neurotoxina o bien la mezcla de neurotoxina o bien la mezcla de neurotoxina está libre de proteínas complejizantes que de manera natural forman los complejos de neurotoxina botulínica junto con las neurotoxinas.

La anterioridad: US 5846929 (folios: 80 a 82), se relaciona con: composiciones que contienen neurotoxina botulínica tipo F, en forma de un producto liofilizado.

La anterioridad: artículo 1, (folios: 83 a 87) trata sobre: inyecciones de toxina botulínica tipo F, que tienen mejor actividad que el botox A.

Estos dos documentos revelan preparaciones farmacéuticas de neurotoxina botulínica -en uno se menciona tipo F y en el otro documento, tipo A-, y se contempla la posibilidad de que dicha neurotoxina esté libre de proteínas complejizantes, tal como se reivindica en la presente solicitud. Además, como se indica en la descripción, dichas preparaciones pueden estar en forma de liofilizado o en forma de inyectable.

Como se observa, el objeto de esta solicitud puede ser idéntico a lo comprendido en el estado de la técnica, con lo cual se considera que podría no cumplir con el requisito de Novedad.

Evaluación de Nivel Inventivo:

El artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina establece que "Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica".

El Nivel Inventivo se evaluó por comparación entre lo solicitado y encontrado en el estado de la técnica y que se relaciona con el objeto reivindicado y de acuerdo con la definición de nivel inventivo del Artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina.

Los documentos **US 5846929** (folios: 80 a 82) y **Artículo 1** (folios: 83 a 87) se relacionan con preparaciones farmacéuticas de neurotoxina botulínica libre de complejos que puedan afectar la actividad de tal sustancia.

Asimismo, revelan que dichas preparaciones pueden emplearse exitosamente para el tratamiento de distintas clases de distonías, y que pueden emplearse otros tipos de neurotoxinas diferentes al tipo A.

La respuesta al problema técnico que se pretendía resolver, resulta obvia partiendo de las anterioridades citadas.

Como se observa, el objeto de esta solicitud podría resultar obvio para una persona del oficio normalmente versada en la materia y derivarse de manera evidente del estado de la técnica.

Evaluación de la Aplicación Industrial:

El artículo 19 de la Decisión 486 de la Comisión de la Comunidad Andina establece que "Se considerará que una invención es susceptible de aplicación industrial, cuando su objeto pueda ser producido o utilizado en cualquier tipo de industria, entendiéndose por industria la referida a cualquier actividad productiva, incluidos los servicios".

El objeto de la presente solicitud cumple con el requisito de Aplicación Industrial.

En conclusión los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

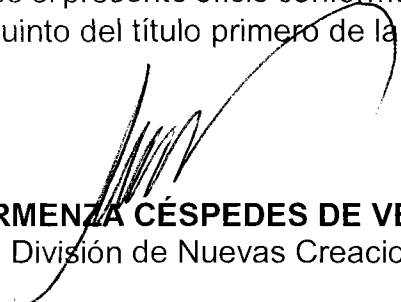
Nº	Documento	Reivindicaciones afectadas	Requisito que afecta
1	US 5846929	1 a 5	Novedad y Nivel Inventivo
2	Artículo 1 ¹	1 a 5	Novedad y Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de la notificación de la presente comunicación. En caso

1 GREENE P., FAHN S. "Use of botulinum toxin type F injections to treat torticollis in patients with immunity to botulinum toxin type A". Dystonia Clinical Research Center. New York (USA); oct. 1993.

de no dar respuesta al presente requerimiento o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha 28 OCT 2011

CONCEPTO TÉCNICO Nº: 1447	EXAMINADOR TÉCNICO Código Nº: 202005	EXAMINADOR JURÍDICO Código Nº:
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