BOTULINUM TOXIN INJECTION AND AIRFLOW STABILITY IN SPASMODIC DYSPHONIA

Running title: Botulinum toxin and airflow stability

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ABSTRACT

Objective: The aim of this study was to analyze the effects of botulinum toxin (BT) injection on airflow stability, by measuring mean phonatory oral airflow and its coefficient of variation (CV), in patients with adductor spasmodic dysphonia (SD).

Methods: Twenty-four patients with SD (aged 31-78 years) and twenty-three controls (aged 29-63 years) were evaluated for mean airflow and its CV during sustained phonation. Fifteen of the patients with SD were also evaluated within three weeks after BT injection.

Results: BT increased airflow in patients ($p = 0.0130$) but neither the preinjection nor postinjection values differed significantly from those of controls. Conversely**,** airflow CV was invariably higher in patients than in controls ($p<0.0001$). But in the 13 patients in whom phonation perceptually improved, including 3 in whom airflow did not increase, airflow CV decreased significantly after BT treatment ($p = 0.0232$).

Conclusion: Patients with SD have highly unstable phonatory airflow; its CV is a valid measure for assessing the outcome of a BT injection. A reduced airflow CV probably does not depend solely on increased airflow due to thyroarytenoid muscle paresis, and may indicate a change in laryngeal motoneuronal activity.

INTRODUCTION

Spasmodic dysphonia (SD) is a disabling motor disorder affecting the voice. Whereas in the past it was diagnosed and treated as a psychiatric disorder, it is currently considered to be a focal dystonia affecting the intrinsic laryngeal muscles. Among the various types described are adductor, abductor, mixed SD and SD with tremor[1-4]. Although patients with SD have a typically strained and strangled voice the grade of voice impairment varies greatly depending on the muscles involved by spasms, the severity of muscle hyperactivity and the compensatory mechanisms during speech. The resulting speech characteristics are therefore influenced by several mechanisms and SD is described as a heterogeneous disorder[3,5].

Whether SD is a single disease or it may arise from various pathophysiologic conditions is unclear. Previous investigations have shown that botulinum toxin (BT) injection into the thyroarytenoid (TA) muscles is the most effective symptomatic treatment for adductor SD[6], as it is for other types of focal dystonia. BT acts peripherally by inhibiting acetylcholine (Ach) release from the presynaptic nerve terminals thereby causing a flaccid paralysis of the injected muscle.

Only few studies have analyzed the aerodynamic features of phonation in patients with SD and described changes after BT treatment of the vocal muscles[7-11]. Although patients with SD would be expected to have a low translaryngeal airflow owing to overclosure of the vocal folds[9,10,12], some investigators report mean airflow within normal limits[7,13].

Because BT acts by paralyzing the TA muscles, BT injections usually increase airflow[7-11]. A limited number of studies have analyzed the variations in airflow during phonation by evaluating its stability over time in patients with SD and assessing changes induced by BT treatment[7,8,12].

In this study we investigated whether aerodynamic evaluation of voice production is useful in the diagnostic assessment and follow-up of patients with SD undergoing BT treatment. To evaluate airflow stability over time, we measured mean oral airflow and its coefficient of variation (CV) during sustained phonation in patients with adductor SD and in a group of control subjects and analyzed changes in both aerodynamic measures in patients after BT treatment.

SUBJECTS AND METHODS

SUBJECTS: We studied 24 patients (19 women, 5 men; age ranging from 31 to 78 years, mean age 54.7) with adductor SD. Severity ranged from mild impairment of fluency to unintelligible speech in the most severe cases. The diagnosis was made by a neurolaryngological team comprising a laryngologist, a speech therapist and a neurologist, and mainly based on the presence of a strained, strangled quality of voice with phonatory breaks and on the patient's clinical history. Flexible videolaryngoscopy confirmed overadduction and spasms of the vocal folds coinciding with the voice stops.

As a control group we enrolled 23 normal subjects (13 women, 10 men; age ranging from 29 to 63 years, mean age 53.4) with no history of voice disorders. The protocol was approved by the hospital ethics committee and all participants gave their informed consent to the study.

BOTULINUM TOXIN TREATMENT: All of the patients received a percutaneous injection of BT type A (Botox) in both TA muscles under electromyographic guidance. Of the 24 patients treated, 18 were receiving their first BT treatment, and 6 were being retreated. BT was injected at a dose of 2.5 mouse units per side in 15 patients and the dose ranged from 0.675 to 5 units per side in the remaining 9 patients, depending on the severity of symptoms or the previous response to treatment, or both. BT was injected at a concentration of 2.5 u/0.1 ml (1.25 u/0.1 ml in patients receiving less than 2.5u per TA muscle).

A*ERODYNAMIC RECORDINGS:* The mean airflow was recorded while patients and controls produced a sustained /a/ at comfortable pitch and intensity for at least 3 seconds, and was measured with EVA2 workstation (SQ-Lab, Aix-en-Provence, France). A mouthpiece was placed on the subject's face, and held tightly around the mouth, to avoid air leaks. The mouthpiece was equipped with built-in acoustic and aerodynamic sensors that were coaxially arranged, to allow simultaneous recordings of acoustic and aerodynamic variables.

Airflow was measured with a constant-temperature mesh pneumotachograph. Voice data were displayed on the computer screen as four superposed curves corresponding to the sound wave, fundamental frequency in Hz, sound pressure level (SPL) intensity in dB, and airflow in dm^3/s. A 1-second segment was then selected for analysis in the steadiest part of the tracings. The EVA2 software was used to compute the acoustic and aerodynamic data. Acoustic results are not considered in this paper. Airflow values were measured in dm^3/s and converted to cc/s.

The CV of airflow, expressed as a percentage, was calculated by dividing the standard deviation (sd) of airflow by the mean airflow:

$$CV = 100 \times \frac{\text{airflow sd}}{\text{mean airflow}}$$

The CV therefore expresses the middle term instability of airflow and can be seen as the scope in % of airflow around its average value. Aerodynamic indexes were assessed before treatment in all patients and in 15 patients the recording was repeated within 3 weeks (range 8-20 days, mean 14.4) after treatment, when the voice improvement had stabilized and breathiness had subsided.

STATISTICAL ANALYSIS: All analyses were performed using the Graphpad Prism statistical program. All values are expressed as means ± sd. The Mann-Whitney test for independent variables was used to compare the mean airflow and the CV values in patients and control subjects. Student's *t*-test for paired data was used to compare mean airflow and CV values before and after BT treatment in the 13 patients achieving voice improvement. A *p* value of <0.05 was considered statistically significant.

RESULTS

AERODYNAMIC RESULTS IN PATIENTS WITH SD / VERSUS NORMAL SUBJECTS

No significant difference was found in mean preinjection airflow values recorded in the 24 patients and 23 controls ($p = 0.0928$) (Figure 1). In both groups mean airflow showed a high degree of variability although 4 patients but only 1 control had airflow values below 50 cc/s. Two patients exhibited values over 300 cc/s (383 and 671 cc/s). The airflow values ranged from 24 to 671 cc/s in the SD patients (mean 145.2 ± 138.0 cc/s) and from 46 to 259 cc/s in the controls (mean 156.0 ± 57.5 cc/s).

Conversely, mean CV of airflow values were significantly higher in patients than in controls ($p< 0.0001$): 64.8 ± 88.3% (range 5.4-329.1%) vs 3.7 ± 2.1% (range 1.2-8.0 %) (Figure 2).

AERODYNAMIC EVALUATION AFTER BT TREATMENT

Aerodynamic recordings were repeated within 3 weeks after treatment in 15 of the 24 patients receiving BT injection in the TA muscles. The remaining 9 patients were followed up only by telephone because they lived far away from our laboratory. Only 13 of the 15 patients who underwent post BT treatment recordings had a subjective and acoustic

improvement in voice quality and fluency. In the 2 unimproved patients postinjection airflow and CV of airflow values were similar to those obtained before treatment.

In the 13 patients who benefitted from BT treatment, mean preinjection and postinjection airflow values differed significantly ($p = 0.0130$), the mean preinjection value being 106.5 ± 61.9 cc/s (range 26-205 cc/s) versus 237.2 ± 138.8 cc/s (range 78-581 cc/s) (Figure 3). In 10 of the 13 patients airflow increased, in 2 it remained unchanged and in the remaining woman patient it significantly diminished (preinjection 182 cc/s versus postinjection 78 cc/s). The difference between postinjection airflow values in patients and controls was borderline but did not reach significance ($p = 0.0652$).

In 12 of the 13 patients in whom BT treatment improved phonation, including one patient in whom airflow also decreased, the mean CV of airflow diminished (preinjection: $48.9 \pm 52.8\%$, range 12.7-213.5% versus postinjection: $9.9 \pm 7.5\%$, range 1.6-27.7%). The decrease was statistically significant ($p = 0.0232$) (Figure 4). Nevertheless the CV values were still significantly higher in treated patients than in controls ($p = 0.0006$).

In the patient in whom airflow decreased after BT treatment, CV diminished from 33.7% to 13.2%.

Figure 5 reports an example of preinjection and postinjection airflow tracings in one of the patients whose voice significantly improved. In this particular case a very high stability of airflow (demonstrated by a significant reduction of CV) was achieved after treatment without a significant quantitative change in airflow.

DISCUSSION AND CONCLUSIONS

The results reported in this study confirm the usefulness of aerodynamic evaluation of phonation in the diagnostic work-up and follow-up of patients with SD, a relatively rare disorder, undergoing treatment with BT.

A few previous papers have measured oral airflow[9-11] and its variability[7,8,12] in SD patients; the results were inconsistent. A low airflow in SD is an expected finding[9,10,12] considering the pathophysiology of the disease, because the overadduction of vocal folds during phonation may well hinder the normal flow of air through the glottis. Nevertheless some authors have reported mean airflow within normal limits[7,13]. The patients we studied, all of whom had adductor SD, had airflow values ranging from 24 to 671cc/s, reflecting the heterogeneity of SD (Figure 1). Airflow values ranged widely also in the control group, confirming previous findings in normal subjects[14]. Patients had lower airflow values than healthy controls though the difference was not significant ($p=0.0928$), probably owing to data dispersion.

Besides reflecting the widely ranging severity of disease, airflow may differ also owing to the various pathophysiological mechanisms. The several different types and subtypes of SD vary depending upon the muscles involved by dystonic activity and also upon other associated neurologic signs and symptoms. A recent study shows[15] that all the intrinsic laryngeal muscles can be involved by dystonic activity in SD. Furthermore, the clinical presentation of adductor SD, the most common laryngeal dystonia, may differ if the disorder is associated with glottic tremor or if the patient uses compensatory mechanisms to avoid voice breaks. Some patients with adductor SD adopt a whispered voice to overcome laryngeal spasms and to be able to communicate; this could explain why 2 patients in our series had very high airflow values (383 and 671 cc/s). Unfortunately neither of these two patients was available for a postinjection recording. Owing to its broad range of values, airflow therefore seems unreliable as a diagnostic test but may be useful for tracking functional changes during follow-up.

After BT treatment mean airflow increased in 10 of the 13 patients (out of the 15 available post-treatment recordings), who experienced a subjective and perceptual voice improvement (Figure 3). The mean postinjection flow value was higher in patients than in normal subjects, but the difference was not statistically significant.

Our results provide further evidence that the high CV of airflow in patients with SD reflects the dystonic activity in the TA muscles during phonation. The patients we studied had remarkably higher CV values than controls before treatment (Figure 2) and values decreased significantly after BT treatment (Figure 4). These results confirm previous findings by others[7,8,11], although our range is wider (5.4-329.1% compared with 21.5-141%, Davis et al[12], and 16.5-146.4%, Finnegan et al[7]). The various series are difficult to compare also because they consider different voice samples. Whereas we used a sustained /a/, others[7,8] analyzed the vowel /i/ in a syllable repetition task and on a shorter segment of voice production.

Although the absolute value of airflow will not discriminate patients with SD from normal subjects, the CV of airflow allowed such a discrimination. Even after BT treatment most of our patients still had significantly higher CV values than controls (Figure 4). Hence, even though the stability of the glottic vibrator improved, it did not return to normal.

In most patients (12 of 13) in whom BT treatment improved phonation the reduced postinjection CV showed that airflow stability increased remarkably. Yet in 2 of these 12 patients airflow remained unchanged and in 1 of the 12 it decreased. The tracings obtained in one of the three patients who had no increase in airflow after BT treatment (Figure 5) clearly show that despite having normal preinjection and postinjection airflow values (167 cc/s and (164 cc/s), she had a markedly reduced CV (from 12.7 to 2.06%). This patient achieved an

excellent voice quality. Only one patient showed a marked reduction in airflow after treatment (see results), though unexpectedly the CV also remarkably decreased. These findings seem to indicate that the higher steadiness of airflow is not entirely due to the flaccid paresis of the TA muscles obtained through BT's peripheral action at the neuromuscular junction. Conversely, they imply that the improved stability of the laryngeal muscular system might arise from a central action of BT. Hence our findings could be in line with Finnegan and colleagues'[7] conclusion , that "increased stability of airflow could reflect […] an increase in stability of the neural signal from the laryngeal motor neuron pool".

Although BT-A decreases muscle hyperactivity in dystonic muscles by preventing Ach release at the neuromuscular junction, some evidence suggests central nervous system (CNS) effects[7,16,17]. When injected into a muscle, BT-A could affect the CNS either by a direct action, through retrograde axonal transport to the central structures, or indirectly, by altering the sensory inputs to the CNS, through a peripheral effect[16,17]. An experimental study[18] has shown that BT injected into the cat gastrocnemius muscle is transported to the spinal cord segments innervating the injected muscle. This study supports the hypothesis that BT may influence spinal cord circuitry through motor and intrafusal afferent axons. Furthermore, when injected into a muscle, BT causes denervation of both the intrafusal and extrafusal fibers[19]. As a consequence, the spindle afferent input could be reduced or altered and ultimately this change might influence the central feedback mechanisms that modulate the activity of laryngeal muscles. Muscles spindles have been described in the intrinsic laryngeal muscles[20], and other mechanoreceptors in laryngeal joints[21]. The sensory feedback deriving from muscles spindles and from the other mechanoreceptors probably influences the activity of the entire laryngeal motoneuron pool. This hypothesis receives support from clinical studies evaluating the effects of unilateral BT injection on the contralateral laryngeal muscles[10,16]. After injecting BT-A into the TA muscle unilaterally, Bielamowicz and Ludlow[16] found that electromyographic activity from both TA and cricothyroid muscles was reduced; they postulated that the achieved voice improvement could be due to both the well-known peripheral and to a central action of BT. Videoendoscopic observations by Zwirner et al[10] showed that unilateral BT treatment reduced the dystonic contraction in laryngeal intrinsic muscles bilaterally.

In our study we treated TA muscles bilaterally. In three patients stability of airflow persisted even when breathiness had subsided and airflow returned to a value similar or even lower than the preinjection one. Why it did so is unclear. SD is a complex disturbance of phonation that probably involves at the same time several laryngeal muscles[2], any of which may be predominant[15]. In their study, Cannito and Johnson[2] defined SD as a "continuum

disorder" meaning that adductor and abductor spasms may both occur with different frequencies in the same patient. Hence we hypothesize that BT injection into TA muscles might also modify the dystonic activity in other laryngeal muscles involved by spasms, either by diffusion or through an indirect effect, by reducing sensory inputs to the CNS.

As a further development of the study, serial recordings of airflow and CV - between consecutive treatments - could be helpful to evaluate whether changes in the two variables are related.

Our data confirm the validity of aerodynamic measures in studying the pathophysiology of spasmodic dysphonia and in monitoring voice changes after BT treatment. Studies of airflow stability may help to explain the possible effects of botulinum toxin on the CNS.

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LEGENDS

Figure 1: The graph reports the values of phonatory oral airflow in the 24 patients with adductor spasmodic dysphonia and 23 normal subjects. Note the wide dispersion of data in both groups. No significant difference was found between the two groups.

Figure 2: Coefficients of variation of airflow in patients with spasmodic dysphonia and controls. Patients' values are significantly higher than those of controls.

Figure 3: Graph showing pre- and post- BT treatment mean oral airflows (airflows) measured in 13 patients with spasmodic dysphonia and in the 23 controls. Although post-treatment values are significantly higher than the pre-treatment values, they do not significantly differ from those of controls.

Figure 4: After botulinum toxin treatment the coefficients of variation of oral airflows are significantly reduced, but they are still significantly higher than those of controls.

Figure 5 A-B: Tracings obtained before (A) and 18 days after (B) botulinum-A treatment in a 31-year-old female patient. The upper tracings are the sound waveforms whereas the lower tracings display the oral airflow (airflow) during the emission of a sustained vowel /a/. Waveforms and airflow tracings are both much more stable in the post-treatment recording. Despite similar airflow values (167 cc/s pre- and 164 cc/s post-treatment), the airflow coefficient of variation has reduced from 12.71% to 2.06%.