Effect of Dietary Zinc and Phytase Supplementation on Botulinum Toxin Treatments

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ABSTRACT

Purpose: To determine whether oral zinc supplementation might affect the efficacy and duration of botulinum toxin treatments.
Methods: In a double-blind, placebo-controlled, crossover pilot study, we examined the efficacy of three botulinum toxin preparations (onabotulinumtoxinA, abobotulinumtoxinA, and rimabotulinumtoxinB) following oral supplementation with zinc citrate 50 mg and phytase 3,000 PU, zinc gluconate 10 mg, or lactulose placebo in individuals treated for cosmetic facial rhytids, benign essential blepharospasm, and hemifacial spasm.
Results: In seventy-seven patients, 92% of subjects supplemented with zinc 50 mg and phytase experienced an average increase in toxin effect duration of nearly 30%, and 84% of participants reported a subjective increase in toxin effect, whereas no significant increase in duration or effect was reported by patients following supplementation with lactulose placebo or 10 mg of zinc gluconate. The dramatic impact of the zinc/phytase supplementation on some patients' lives clinically unmasked the study and prompted an early termination.
Conclusions: This study suggests a potentially meaningful role for zinc and/or phytase supplementation in increasing the degree and duration of botulinum toxin effect in the treatment of cosmetic facial rhytids, benign essential blepharospasm, and hemifacial spasm.

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INTRODUCTION

Botulinum toxins (BTXs), a family of zinc-dependent metalloproteases, play an expanding role in the management of many medical and aesthetic conditions. While generally effective for most patients, BTX therapeutic efficacy is well known to vary widely, not only among individuals, but also from treatment to treatment for a single individual, and certain demographic variables, such as age over 64 years, are associated with a profoundly diminished BTX effect.[1,7,8,13]

Due to the obligate requirement of zinc for BTX function, and the fact that commercial preparations of BTXs exclude zinc addition, a person's zinc status may be an important variable in BTX clinical effect.

Profound zinc deficiency in childhood can be blatant,[24] but less severe deficiencies in adulthood may be subtle. While the exact prevalence of zinc deficiency is unknown due in large part to poor correlation of serum and urine levels with tissue and intracellular concentrations and overall elemental zinc effect,[11,28] concern over suspected widespread marginal zinc status is growing.[5,17,20,26] For example, in the United States (U.S.), roughly half of persons over age 50 consume less zinc than federally recommended,[4] and nearly 30% of these individuals may show overt signs of zinc deficiency.[23]

Zinc levels are dependent upon many factors aside from zinc dietary intake including dietary phytates (TABLE 1), a family of phosphorous-containing compounds that block zinc absorption (TABLE 2).[18] A relatively low zinc/high phytate diet may be increasingly prevalent in the U.S., especially among fixed-budget elderly avoiding expensive meats, the weight- and cholesterol-conscious, and vegetarians. Phytases, a family of enzymes that degrade phytates, are known to increase zinc absorption when consumed with zinc and phytates.[2,15]

Zinc homeostatic mechanisms maintain tissue levels for only brief periods, and individuals may become relatively zinc deficient quickly.[14] Although the effects of BTX treatments may last months, the zinc-dependent proteolytic activity of BTXs occurs before the toxins are degraded within hours of administration.

TABLE 1.

Factors Adversely Affecting Zinc Levels
Dietary
Low zinc
vegetarianism
red meat avoidance
High phytate (see Table 2)
Alcohol
Casein (milk-based products)
Phosphates (soft drinks)
EDTA preservative (in many foods)
Supplements
poorly absorbed forms of zinc
high fiber
Vitamin A
Iron
Calcium[a]
Copper, magnesium
Age
< 18 years[b]
> 50 years[c]
Increased Metabolic Demand
Pregnancy
Acute Illness (burns, infections)
Malabsorption
Frequent diarrhea
Frequent laxative use
Gastrointestinal maladies

Factors Adversely Affecting Zinc Levels. a = contentious, b = deficiency more common in certain subgroups, c = studies vary in definition of "elderly" some report on groups over age 50 and others over age 65.

Thus, to achieve BTX effect, tissue zinc levels in recipients must be adequate merely around the time of toxin injection.

Rising concern over general zinc status both globally and in the U.S. prompts the question of whether zinc availability might be one factor responsible for variation in BTX effect. Since accurate measure of transient zinc levels in question can be obtained only by the impractical biopsy of facial neuromuscular tissue, and chronic elevation of zinc may lead to chronic zinc toxicity, we wondered whether arbitrary phytase-assisted, oral zinc loading just prior to BTX treatment might diminish the variability of BTXs effect.

The purpose of this double-blind, placebo-controlled, crossover pilot study was to determine whether phytase-assisted oral zinc intake might affect the efficacy of BTX treatments in patients routinely being treated for cosmetic facial rhytids (CFR), benign essential blepharospasm (BEB), or hemifacial spasm (HFS).

TABLE 2.

Dietary Zinc and Phytate Content		
Dietary Source	Zinc (mg/100g)	Phytate (mg/100g)
Oysters	9.0-74.7	0
Turkey	2.1-6.4	0
Beef, Pork	2.9-4.7	0
Chicken	0.9-3.4	0
Eggs	1.1-1.4	0
Fruits	0-0.2	0-63
Peas	0.8	28
White Bread	0.9	30
Oatmeal, Oats	0.5	111
Chickpeas	1.4	208
Vegetables	0.1-0.8	30-439
Beans, Lentils	1.0-2.0	100-617
Whole Grains	0.5-3.2	211-618
Peanut Butter	2.9	1,252
Soy	0-0.2	800-1,750
Wheat, Cereals	2.3	1.467
Seeds, Nuts	2.9-7.8	176-4,710

Zinc and Phytate Content in Foods.[6, 10, 12, 21, 22]

METHODS AND MATERIALS

Study Design

In this double-blind, randomized, placebo-controlled, inter-treatment washout, crossover pilot study, we compared the effectiveness of BTX injections: Botox (onabotulinumtoxinA, Allergan, Irvine, CA); Myobloc (rimabotulinumtoxinB, Solstice Neurosciences, Malvern, PA); Dysport (abobotulinumtoxinA Tercica, Brisbane, CA) after four days of supplementation with either oral zinc citrate 50 mg and phytase 3,000 PU (Z50), zinc gluconate 10 mg (Z10, a dose roughly equivalent to the U.S. federally recommended daily intake), or lactulose placebo (P).

Study Participants

Patients selected for this study from the senior author's practice all had well-established treatment protocols with individualized BTX injection frequencies, patterns, doses, and toxin types maximized for efficacy for each patient in the treatment of benign essential blepharospasm (BEB), a dystonia causing forceful eyelid spasm and eye closure, hemifacial spasm (HFS), or cosmetic facial rhytids (CFR), and met the inclusion criteria in Table 3.

For purposes of analysis, BEB patients were classified as "hard to treat" (BEB_H) or "easy to treat" (BEB_C) (TABLE 3). BEB_C patients were highly consistent, successful toxin responders, whereas BEB_H patients were those who had failed to achieve any ben-

TABLE 3.

Study Inclusion Criteria
> 18 years old
• Ability to understand and sign consent form
• Non-pregnant
• Completion of a dietary history questionnaire and ability to maintain pre-study diet
• Ability to comply with study protocol and keep follow-up visits
• Reliable completion of weekly (or daily)† Effect Diary
• Absence of chronic gastrointestinal disease (frequent diarrhea, Sprue, or inflammatory bowel disease)
• Minimum of 3 (or 5)‡ prior, unchanging injection patterns with the same botulinum toxin for the treatment of BEB, HFS, or CFR
• More than 50 unit equivalents of Botox brand botulinum toxin per treatment‡
• Patient regularly perceived suboptimal treatment results, despite maximal therapy‡
• Failed attempts by at least two other physicians to achieve toxin injection benefit‡

Study Inclusion Criteria. † BEB patients completed Effect Diaries daily, whereas CFR and HFS patients completed them weekly. ‡required for "hard to treat" categorization of Benign Essential Blepharospasm. Unit equivalents of Botox brand botulinum toxin were as follows: 50 units Myobloc = 2.8 units Dysport = 1 unit Botox.

TABLE 4.

Botulinum Toxin Efficacy Grading Scale	
Score	Interpretation
-3	Worst effect ever or imaginable
-2	Significantly less effective than usual
-1	Slightly less effective than usual
0	No change from usual range of effect
1	Slightly more effective than usual
2	Significantly more effective than usual
3	Best effect ever or imaginable

Botulinum Toxin Efficacy Grading Scale. Patient subjective assessments of toxin effect recorded on a daily basis for blepharospasm patients and on a weekly basis for all others.

efit from BTX therapy from at least two other physicians prior to being treated by the senior author, never achieved full, normal daily function despite maximal medical, surgical, and BTX treatments, and received more than 50 Botox brand equivalence units (50 units Myobloc = 2.8 units Dysport = 1 unit Botox) per treatment (to exclude patients with isolated pseudoapraxic BEB).

Assignment, Treatment, and Cross-over

Participants were assigned in a double-blinded fashion to one of three supplements (P, Z10, or Z50) provided in identical-appearing

TABLE 5.

Indication for Patient Treatment				
Treatment Indication	BEB_H	BEB_C	HFS	CFR
Total Participants	30	27	8	33
Number of Z50 Participants	25	20	5	27
% Female†	80%	75%	20%	80%
Average Age† (years)	74.6	64.5	55.8	50.4
Number Termed	5	7	3	6

Indication for Patient Treatment. BEB_H = "hard to treat" Benign Essential Blepharospasm, BEB_C = "easy to treat" Benign Essential Blepharospasm, HFS = hemifacial spasm, and CFR = cosmetic facial rhytids. "Total Participants" = number of all participants for each treatment indication. "Number Z50 Participants" = number of patients who received Z50 and at least one other treatment arm. † = among participants who received Z50 supplementation. "Number Termed = number of patients who never received Z50 due to study termination

powder-filled, gelatin capsules (Green Park Pharmacy, Houston, Texas) to be taken for four days prior to BTX injections administered by the senior author in accordance with each participant's established individualized toxin, dose, and treatment pattern. After completing the initial treatment, patients underwent a "wash-out" where they obtained their usual injection treatment without any supplement, then crossed over into the next double-blinded treatment arm. This was repeated a second time so that patients participated in all 3 arms of the study in random order.

Follow-up and Outcome Measures

All BEB study participants kept a daily log of BTX effect, documenting their ability to keep their eyes open and perform daily activities. Participants with CFR or HFS kept a weekly log of treatment effect. Patients were required to show their logs upon return visits. Using these logs and established treatment experiences prior to enrollment in the study as well as during "wash-out" phases, participants graded their overall treatment efficacy for each arm of the study on a scale of -3 to +3 (TABLE 4), and patient-reported change in duration of effect, as documented in logs, was calculated as the ratio of study treatment duration over pre-study treatment duration, less one.

Statistical Analysis

Statistical analysis was performed using STATA 11 (StataCorp LP, College Station, Texas) with analysis of differences in effect duration performed using ANOVA one-way analysis of variance and Sidak post-hoc tests. Differences in efficacy were analyzed using chi-squared tests. Data from study participants who took all three supplements were analyzed using the Friedman two-way ANOVA and Cochran's Q test. The Z50 study results were divided into subgroups based upon age (<65 and ≥65 years), gender, the indication for treatment (BEB_H, BEB_C, HS, or CFR), and type of BTX used and analyzed with the chi-square test (age, gender), and Kruskall-Wallis equality-of-populations rank test and Fisher's exact test (indication for treatment and type of

FIGURE 1. Grey bars indicate average increase in toxin effect duration across all patients receiving one of three supplements. The blue bar indicates the average increase in toxin effect duration among the 92% of patients who reported an increase.

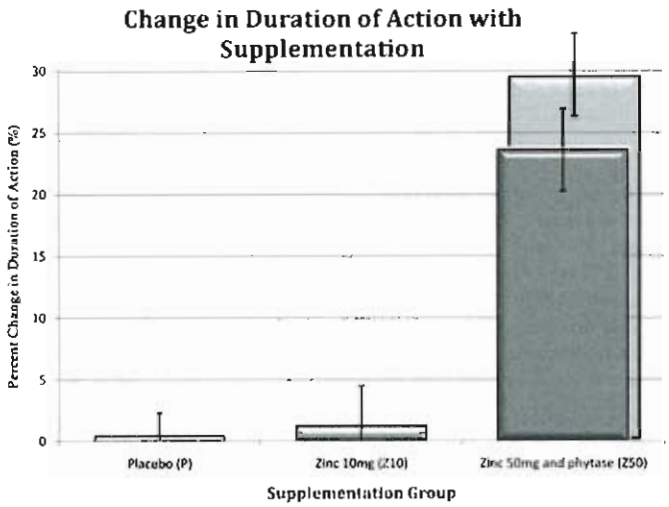
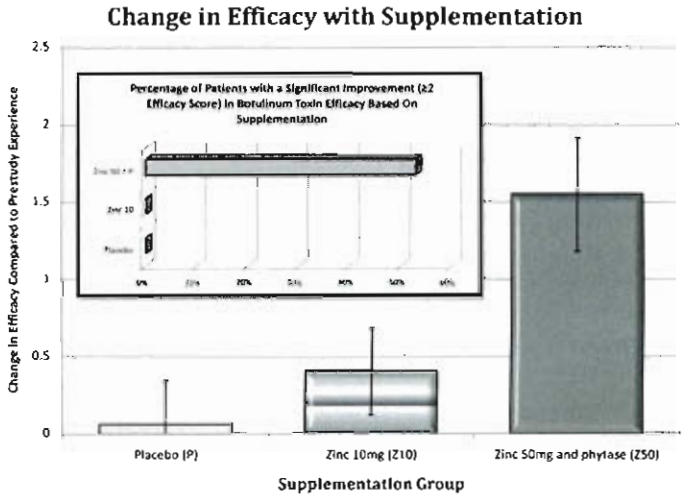


FIGURE 2. Vertical grey bars indicate the average perceived increased efficacy of toxin treatment by supplement type taken. Efficacy scoring per Table 2. Inset shows that in the Z50 group, over 50% reported a "significant" = 2 or "best possible" = 3 increase in effect.



BTX used). Relationships between demographics and benefit were examined using multivariate linear regression and ordinary least squares method.

RESULTS

Participants

From November, 2007 to December, 2010, a total of 98 participants (77 female) were enrolled from the senior author's practice where they had been receiving BTX injections for an average of 7 years (range 9 months to 23 years).

Follow-up, Compliance, and Early Study Termination

No patient was lost to follow-up and all reported 100% supplement usage compliance and demonstrated 100% diary completion compliance. However, during the study, many of the BEB_H patients reported life-altering effects, essentially clinically unmasking the study. For example, a 36-year-old woman diagnosed with BEB 6 years earlier and who failed BTX treatments by 7 other physicians was driven 450 miles each way by her family for BTX treatments from the senior author every three months for 18 months with only modest improvement, allowing her to keep her eyes open enough to ambulate around her home and cook, but not drive to the grocery store. After entering a second arm of the study, she appeared for her next injection having driven the 450 miles herself, demanding an unmasking of the study and more of the same supplement.

Based upon the life-altering, remarkable experience of many patients, the data were prematurely unmasked and the pilot study terminated. Thus, although 98 patients were initially enrolled, only 77 received Z50 supplementation; all of those who did also received at least one other study arm supplement. Twenty-seven patients completed all three study arms, 24 completed Z10 and Z50 study arms, and 26 completed P and Z50 study arms. Treatment indications for these patients are shown in Table 5.

Comparisons Between Study Supplements

Duration of toxin effect was significantly greater following Z50 supplementation compared to either the Z10 or P supplementation (average = 29.6%, range 0.12 - 1.15% increase, $P<0.001$, (FIGURE 1). Efficacy ratings were also significantly greater ($P<0.001$) for the Z50 group with increased effect reported by 84% (53% with "significant" improvement rating of 2 or 3) compared to both the Z10 and P groups (FIGURE 2). The patients who underwent supplements in only the Z10 and/or P treatment arms showed no significant difference from pre-treatment experiences ($P= 0.8835$).

Impact of Demographic Variables

Individuals 65 years or older receiving Z50 supplementation were more likely to report improvement in the degree of toxin efficacy (79% vs. 29%, $P<0.0001$), yet age did not influence toxin effect duration. No association was seen between gender and degree or duration of effect ($P=0.9895$ and $P=0.4135$, respectively).

Following Z50 supplementation, patients treated for HFS showed a greater increase in toxin effect duration compared to those treated for BEB_C and BEB_H ($P<0.05$), but not for CFR ($P=0.0566$). Conversely, BEB_H patients were most likely to report an increase in treatment efficacy (88%), whereas CFR patients were least likely (27%) ($P<0.001$ for both).

AbobotulinumtoxinA was found to be less likely to result in patient-reported increase in toxin efficacy than onabotulinumtoxinA and rimabotulinumtoxinB ($P=0.03$).

Safety

Despite receiving their usual toxin doses and patterns, five subjects (6.1%; all females; mean age 64 years) in the Z50 study arm experienced too much BTX effect and scored their efficacy as -2 or -3. Four BEB patients (BEB_C, n=3; BEB_H, n=1) suffered lagophthalmos, successfully medically managed. One CFR patient experienced more brow akinisis than desired.

DISCUSSION

Botulinum toxins (BTXs) commonly used for both functional and aesthetic conditions such as cosmetic facial rhytids (CFR), Benign Essential Blepharospasm (BEB), and hemifacial spasm (HFS), show variability in treatment effect and duration both among subjects and between different treatment sessions for a single individual.[1,7,8,13] Given BTXs obligate requirement for elemental zinc and studies suggesting wide-spread zinc deficiency across the U.S. and the world, we wondered whether a relative zinc deficiency might be another important variable.

Using individualized, patient-specific, established injection doses and patterns as well as toxin types and brands preferred by each patient, participants in the study were able to compare pre-study experiences with each study treatment arm as well as with inter-treatment arm "washout" period injections.

Across all three treatment indications, after taking the Z50 supplement, 92% of patients reported an average 30% increase in BTX effect duration and 84% claimed an increase in toxin efficacy with more than half stating the difference was "significant." For many BEB_H patients, the effect was so clinically important that it made the difference between being functionally blind and homebound versus being able to read, watch television, drive, grocery shop, and use a computer, thus effectively unmasking the study, and causing patients to withdraw and demand more Z50 supplementation.

Following Z50 supplementation, patients treated for HFS showed a slightly greater increase in toxin effect duration than BEB patients ($P<0.05$), which may be the result of the often-diminished facial nerve function in HFS.[19,27]

Subjects 65 years or older taking the Z50 supplements were more than 2.5 times as likely as younger patients to report a "significant" change in toxin efficacy, correlating prior reports of both diminished BTX effect and diminished zinc levels in older individuals.[1] BEB_H patients were also more likely to report a significant increase in toxin effect than BEB_C, HFS, and CFR patients (88%, 47%, 40% and 27%, respectively), probably reflecting that BEB_H patients, by definition, were generally less pleased with their toxin results prior to the study, whereas CFR patients were quite happy with their pre-study treatments.

The benefits of Z50 supplementation did not appear to differ among BTX formulations studied. This is not surprising as all of the toxins undergo similar polypeptide processing, cell binding and uptake, light chain activation, and zinc cofactor binding to achieve proteolytic function.[3,25]

Further observations after the termination of this study (data not presented) suggest that repeated Z50 supplementation often decreases treatment-to-treatment variability for each patient, potentially due to moderating regular fluctuations in zinc levels. Additionally, increased BTX efficacy for many study participants has translated into using less BTX to achieve their same treatment goals. A reduction in toxin dose and frequency may be also beneficial in terms of recent concerns regarding potential dose-dependent distant spread of BTX.[9,16]

This pilot study raises many questions. First, it is not clear whether the benefits seen with Z50 supplementation were due to the specific dose of zinc, the phytase, the citrate, or some combination. For acquisition convenience, zinc gluconate was used in the 10 mg treatment arm, whereas zinc citrate was used in the 50 mg treatment arm. Although many suggest there is no difference in zinc absorption between these two preparations, this may be a confounder. It is also not clear that 50 mg zinc citrate and 3,000 IU phytase four days in advance of toxin injections is the optimum supplementation for everyone, or indeed for anyone.

Second, zinc levels in the facial musculature was not studied to determine whether zinc levels rose or not, and molecular and physiologic studies were not performed to determine whether the added zinc actually increased toxin-zinc binding and activation in vivo, or whether the zinc, citrate, or phytase acted on muscles or nerves synergistically with local toxin injections to achieve the observed effects. All that can truly be said is that the Z50 preparation used increased both toxin effect and duration in the majority of patients tested.

Finally, all outcomes in this study were patient-reported and subjective. Although the very low placebo effect suggests good patient objectivity, some readers may find neuromuscular stimulation data to be more objective and compelling.

CONCLUSIONS

This pilot study suggests an important role for combined zinc and phytase supplementation in increasing the duration and efficacy of botulinum toxins in patients treated for cosmetic facial rhytids, benign essential blepharospasm, or hemifacial spasm, potentially reducing the number of treatments per year, the total dose of toxin per treatment, and inter-treatment variability for individuals. It is also possible that patients who were previously poor- or non-responders to botulinum toxin therapy may recognize new benefit with zinc and phytase supplementation. Further multi-center, dose-dependent studies are indicated.

DISCLOSURES

Dr. Soparkar has a patent pending on the use of zinc and phytase combination for botulinum toxin efficacy enhancement.

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