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Research paper

Botulinum toxin A (BoNT/A) for the treatment of depression: A randomized, double-blind, placebo, controlled trial in China

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ABSTRACT

Background: Depression is characterized by low moods, anhedonia, and social avoidance. Effective and acceptable treatments are required for depression. Positive effects on mood have been observed in patients with depression after treatment with botulinum toxin A (BoNT/A).

Methods: A total of 88 patients with depression were randomly assigned to BoNT/A (n = 61) and placebo (saline, n = 27) groups. The primary objective was to determine the change in the 17-item version of the Hamilton Depression Rating Scale (HAMD), 12 weeks after the treatments when compared with the baseline.

Results: The BoNT/A and placebo groups did not differ significantly in all the collected baseline characteristics. However, there was a significant improvement in the depressive symptoms of the BoNT/A group compared to those of the placebo group throughout the 12-week follow-up period. This was according to the measurements of HAMD ($F(1, 370) = 9.094$, $P = 0.0027$), Self-rating Depression Scale (SDS) ($F(1, 370) = 11.26$, $P < 0.001$), Hamilton Anxiety Scale (HAMA) ($F(1, 410) = 8.673$, $P = 0.0034$) and Self-rating Anxiety Scale (SAS) ($F(1, 379) = 5.788$, $P = 0.017$). Furthermore, the effectiveness was even higher at the end of the study period.

Limitations: The limitations include the absence of a multicenter study and an inadequate number of cases. Additionally, the mechanism of BoNT/A antidepression was not studied.

Conclusion: This study showed that a single treatment with BoNT/A may accomplish a strong and sustained alleviation of depression in patients.

1. Introduction

Depression is the commonest mental disorder and characterized by low mood, anhedonia, and social avoidance (Li et al., 2021b). It is classified into minor, moderate, and severe disease. Subthreshold depression (minor depression) is generally defined as a depressive state failing to reach the diagnostic threshold for major depression, which is highly prevalent, clinically relevant, and societally important (Li et al., 2020). According to the World Health Organization, depression affected >264 million people globally in January 2020 (Erchinger et al., 2021). Prevalence rates of minor depression vary from 0.7 % to 6.8 % for the general population (Pfeil et al., 2017). Depression presents a heavy burden to the family of the patient and the healthcare system. It is commonly underdiagnosed and less treated, because of its subjectivity (Bess et al., 2013). However, higher screening rates could enhance depression diagnosis and treatment (Samples et al., 2020). Among the patients diagnosed with primary anxiety disorders, 63 % are current and 81 % are lifetime depressive disorder diagnoses (Groen et al., 2020). However, depressive and anxiety disorders are closely related.

Although various therapies for depression have been developed, reports indicate that 30 % of patients have developed resistant depression, which cannot be treated with traditional antidepressant medications such as selective serotonin reuptake inhibitors (SSRIs) (McIntyre et al., 2014). Additionally, approximately one-third of patients with depression fail to respond to the existing pharmacotherapy (Dong et al., 2017). Further, conventional antidepressants are associated with a delayed

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onset of action. Moreover, they have several side effects such as gastrointestinal discomfort, cognitive decline, and constipation (Huang et al., 2017). Therefore, there is an urgent need for researchers to seek and develop better options for treating depression, especially minor depression, which may worsen and develop into severe depression (Jiang et al., 2019).

Recently, increasing evidence suggests that Botulinum toxin A (BoNT/A) has significant effects in the treatment of depression. Further, several randomized controlled trial (RCT) studies have demonstrated the great efficacy of BoNT/A as an antidepressant (Li et al., 2021a). Local injections of BoNT/A have been found to relax the muscle by cleaving synaptosomal associated protein-25 kD (SNAP-25) at the neuromuscular junction. Synaptosomal-associated protein-25 kD is a soluble NSF-attachment protein receptor (SNARE) protein, which is critical for synaptic vesicle fusion to the inner surface of the cellular membrane (Rossetto et al., 2014). Local injection of BoNT/A up-regulates the level of brain-derived neurotrophic factor (BDNF) in the hippocampus and activates the downstream ERK-CREB signaling pathways in stressed mice (Li et al., 2019). There are different hypotheses for the antidepressant properties of BoNT/A, and the facial feedback hypothesis is one of the top three (Li et al., 2019). However, several scholars also support the reverse transport hypothesis (Cai et al., 2017; Mazzocchio and Caleo, 2015). The two hypotheses explained the potential anti-depression mechanisms of BoNT/A from macroscopic and local fields.

Previous studies indicate that a single session of therapeutic injections of BoNT/A into facial muscles in the glabellar region may represent a novel, well-tolerated treatment option for depression (Finzi and Rosenthal, 2014; Finzi and Wasserman, 2006; Magid et al., 2014; Wollmer et al., 2012). Botulinum toxin A corrugator and procerus injections have an acceptable record of safety (Brin et al., 2009). In the published depression trials, the common treatment-emergent adverse effects of BoNT/A were only temporary and local to the treatment site (headache and injection site irritation) (Finzi and Rosenthal, 2014; Wollmer et al., 2012). The findings of various studies indicate that BoNT/A may produce antidepressant effects lasting several months following a single treatment session, and this may increase treatment adherence compared to daily medication. However, these previous studies focused on major depressive disorder and non-Chinese patients. Therefore, this study aimed to evaluate the efficacy, safety, and duration of the effect of a single treatment session of BoNT/A on patients with minor and moderate depression in general hospitals in China.

2. Materials and methods

2.1. Patients

This was a 12-week multicenter randomized, double-blind placebo-controlled study. It had 100 units (U) of BoNT/A (Hengli, Cat. S10970037, Lanzhou, China) or placebo in patients with minor or moderate depression. We enrolled patients with minor or moderate depression who were diagnosed in the Second Affiliated Hospital of Soochow University between January 2019 and January 2021. The patients were in a minor or moderate depressive episode lasting $\geq$2 weeks. The basic information of all participants was recorded, including age, gender, previous medical history, and education level. All enrolled patients and their families signed informed consent forms before the study. An overview of this study is illustrated in Fig. 1.

Inclusion criteria of this study included: (1) The patients' diagnoses were consistent with the standard of depression in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) for depression. Briefly, symptoms include low mood, loss of interest and pleasure, lack of energy, fatigue, decreased attention, decreased self-evaluation, self-guilt, and feelings of worthlessness, pessimism, self-injury/suicide thoughts or behaviors, sleep disorders, and loss of appetite. And, the course of the disease was >2 weeks. (2) All the patients were aged 18 to 75 years; (3) The score of the 17-item Hamilton Depression Scale (HAMD-17) (Hamilton, 1960): $7 \leq$ HAMD-17 ≤ 24; (4) Patient signs agreement to participate in this program.

Conversely, the exclusion criteria were: (1) Patients with psychiatric disorders other than depression; (2) Patients with other systemic diseases and brain organic diseases caused by depression; (3) Patients with a history of head disease, brain injury, epilepsy, and other nervous system diseases; (4) Patients with a history of severe cardiovascular system disorders, as well as respiratory, immune, and systemic disease history; (5) Patients with a drug history of antipsychotics, antidepressants, sedative medications, alcohol, morphine, or drug abuse; (6) Patients with liver and kidney dysfunction; (7) Inability to cooperate with

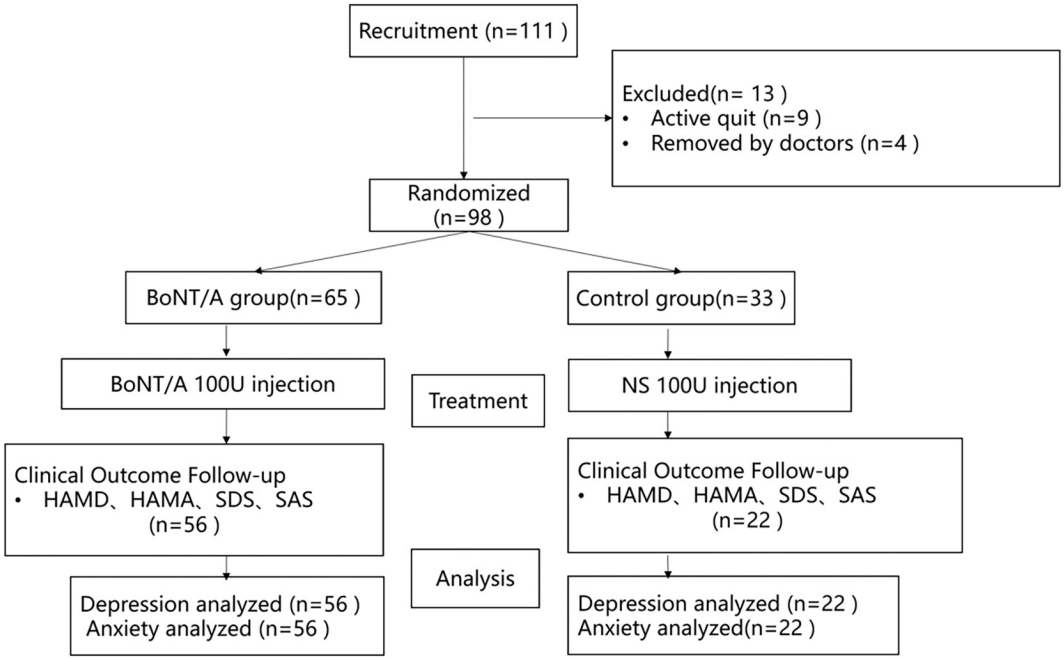

Fig. 1. The study road map of the experiment.

the completion of the relevant neuropsychological tests; (8) Lactating or pregnant women.

2.2. Drugs and administration

The 100 U BoNT/A or placebo was diluted with 0.9 % saline, making a final volume of 2 mL. According to our previous study (Zhang et al., 2021), 10 points were injected at once into the frowning muscle, depressor muscle, and occipital frontalis muscle. In addition, five points were chosen for the injection of BoNT/A, each at the lateral canthus of the eyes and the bilateral temporal region. A total of 20 sites and 5 units per site were applied for BoNT/A (Fig. 2). Reconstituted study medications that were not administered immediately were kept in the vial (stored at 2–8 °C), and vials that were not used within 4 h were discarded.

2.3. Blinding and randomizing

The packaging and content were kept consistent in the both drugs to maintain blinding. Drugs were numbered and randomized into different experimental groups by the medical provider. The number edited on the bottle corresponded to the drug that was released at the end of the trial. The treatment and control group proportions were in a ratio of 2:1. During the treatment, the person who administered the injection was different from the evaluator. The evaluators included several persons, who were trained uniformly. The patient will use a hat to cover the area above the eyes during the scale assessment.

2.4. Assessments and safety

After randomization and treatment, all patients were followed up in an outpatient clinic at weeks 1, 2, 4, 8, and 12 using the HAMD, 14-item Hamilton Anxiety Scale (HAMA-14), Self-rating Depression Scale (SDS), and Self-rating Anxiety Scale (SAS). Patients were discontinued from the assessment if they required concomitant medication for depression or a modification in their cognitive therapy regimen (from screening) at any time during the study. Patients were also observed for any allergic reactions for the first 0.5 h after the treatment period. Further, there were sustained communications during the test periods.

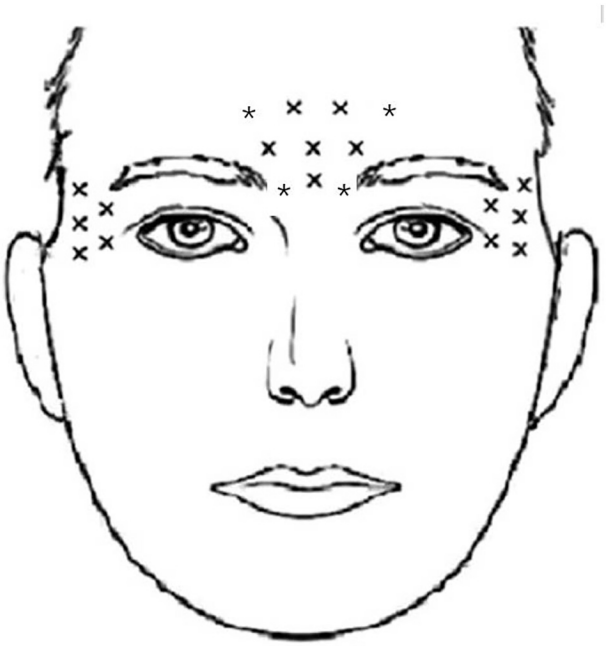

Fig. 2. Schematic representation of BoNT/A injection sites. * & X stands for the injection site, 10 points for frowning muscle, depressor muscle, and occipital frontalis muscle; five points each for lateral canthus and the bilateral temporal region. * Points are taken from the literature (Finzi and Rosenthal, 2014). X Points are added points. A total of 20 sites and five units per site were applied for the BoNT/A.

2.5. Ethics approval

This study was a placebo, double-blind randomized controlled study. All the protocols were approved by the Medical Ethics Committee of the Second Affiliated Hospital of Suzhou University (number: JD-LK-2017-011-02). Moreover, this study was registered in the Chinese Clinical Trial Registry (ChiCTR1800019802).

2.6. Statistical analysis

All data were analyzed using the GraphPad Prism 8.3 statistical software. P-values < 0.05 were considered significant. The HAMD, HAMA, SDS, and SAS scores before and after treatment with BoNT/A or placebo were compared by two-way repeated-measured ANOVA. Results were presented as mean ± SEM.

3. Results

3.1. Patient characteristics

The population consisted of 98 patients who received treatment and were randomized as follows: BoNT/A 100 U (n = 65) and placebo 100 U (n = 33). Of the 98 patients, 78 patients completed all 12 weeks, while 10 patients were lost to follow-up, and another 10 patients missed some weeks of follow-up because of the action control of COVID-19 and personal reasons. Demographic characteristics included gender, age, marriage, education, residence, and family structure. Patient baseline characteristics are summarized in Table 1.

3.2. Efficacy assessments

3.2.1. BoNT/A showed antidepressant efficacy

After 12 weeks of follow-up, decreased HAMD scores were observed in both groups compared with the baseline (Fig. 3A. F_{time} (4, 380) = 26.93, $P < 0.001$). Similar results were found in SDS (Fig. 3B. F_{time} (4, 380) = 9.08, $P < 0.001$). The endpoint for observation was 12 weeks. At this point, the mean HAMD scores in the placebo and BoNT/A groups decreased to 8.33 and 5.78, respectively. Meanwhile, the mean SDS

Table 1
Baseline characteristics of patient groups.

Group	BoNT/A	Control	Statistics	P
Number	56	22	–	–
Gender (male:female)	18:38	6:16	0.176	0.675
Age (years)	53.86 ± 15.142	55.50 ± 13.215	−0.446	0.657
Marriage (1/2/3/4, %)	91.1/7.1/0/1.8	95.5/0/4.5/0	3.789	0.347
Education (a/b/c/d, %)	17.9/23.2/30.4/28.6	13.6/31.8/36.4/18.2	1.466	0.750
Residence (city: village)	51:5	18:4	0.573	0.449
Family structure (i/ii/iii, %)	60.7/39.3/0	68.2/31.8/0	0.377	0.539
HAMD baseline	12.82 ± 4.553	13.00 ± 3.830	−0.163	0.871
HAMA baseline	13.25 ± 5.737	14.00 ± 4.557	−0.548	0.585

1/2/3/4: married/unmarried/divorced/widowed.
a/b/c/d: illiteracy/primary school/high school/university and above.
i/ii/iii: living with children/live alone/living in a nursing home.
Normally distributed data are expressed as mean ± standard deviation, and independent sample *t*-test is used for comparison between groups; Chi-square test/Fisher's exact test is used for measurement data comparison; $P < 0.05$ indicates a statistical difference.

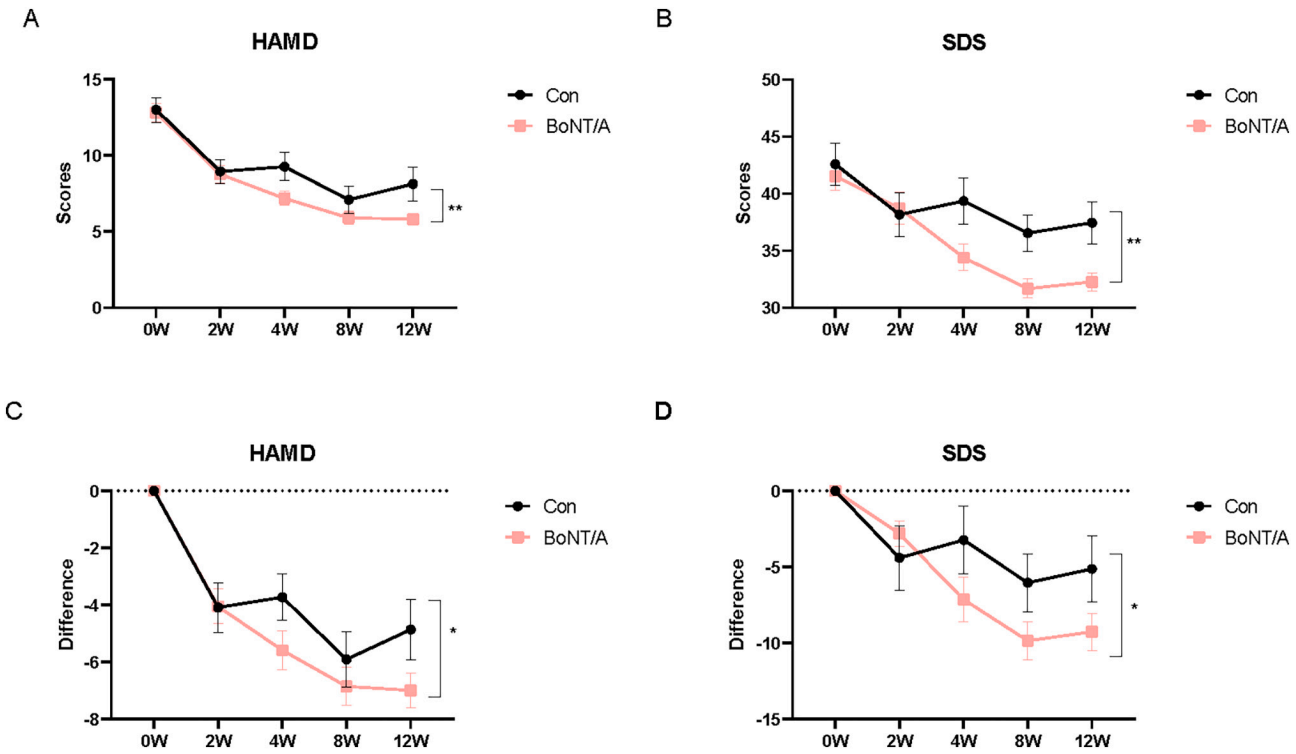

Fig. 3. Changes in HAMD and SDS scores for 100 U treatments.
The changes in the HAMD and SDS scores between the BoNT/A and control groups are shown in A and B. The changes in the HAMD and SDS scores from the baseline between the BoNT/A and control groups are shown in C and D. The difference between the BoNT/A and sertraline groups at each time point was determined using two-way repeated-measured ANOVA. *: there was statistical significance between the control and BoNT/A group, $P < 0.05$. Con, control, n = 22; BoNT/A, botulinum toxin A, n = 56; HAMD, Hamilton Depression Scale; SDS, Self-Rating Depression Scale; W, week. Data are summarized as mean ± SEM.

scores in the placebo and BoNT/A groups decreased to 38.05 and 32.25, respectively. The effectiveness of BoNT/A was higher than that of the placebo, according to the changes in the HAMD scores (Fig. 3A. $F_{drugs}(1, 380) = 7.41$, $P = 0.0068$) and SDS (Fig. 3B. $F_{drugs}(1, 380) = 10.86$, $P = 0.0011$). The changes in the effectiveness of the different treatments from the baseline were also evaluated to make the effects more visible. The results of this study showed that BoNT/A can decrease the HAMD and SDS scores more significantly in comparison with the placebo (Fig. 3C. $F_{drugs}(1, 380) = 4.36$, $P = 0.037$; Fig. 3D. $F_{drugs}(1, 380) = 4.77$, $P = 0.03$). Therefore, these results suggest that BoNT/A has appreciable antidepressant potential.

3.2.2. *BoNT/A showed anti-anxiety efficacy*

After 12 weeks of follow-up, the HAMA scores were decreased in the two experimental groups when compared with the baseline (Fig. 4A. $F_{time}(4, 410) = 18.8$, $P < 0.001$). Similar results were reported for the SAS (Fig. 4B. $F_{time}(4, 379) = 12.68$, $P < 0.001$). At the end of the observation period (12 weeks), the mean HAMA scores decreased to 8.09 and 7.15 in the placebo and BoNT/A groups, respectively. However, the mean SAS scores decreased to 37.23 and 33.57 for the placebo and BoNT/A groups, respectively. Treatment of depression with BoNT/A had greater effectiveness than that of the placebo, according to the difference in the HAMA scores (Fig. 4A. $F_{drugs}(1, 410) = 8.673$, $P = 0.0034$) and the SAS (Fig. 4B. $F_{drugs}(1, 379) = 5.788$, $P = 0.017$). To make the effects more visible, the changes in the effects of the two treatments were observed as compared with the baseline. The results showed that BoNT/A significantly decreased the SAS scores, compared with the placebo group (Fig. 4D. $F_{drugs}(1, 380) = 9.097$, $P = 0.0027$). However, BoNT/A did not significantly decrease the HAMA scores (Fig. 4C. $F_{drugs}(1, 380) = 2.011$, $P = 0.1570$). Therefore, these results suggest that BoNT/A has a remarkable anti-anxiety effect.

3.2.3. *Additional information*

Four patients were withdrawn from this study because they failed to follow the doctor's instructions. The withdrawal rates were 4.9 % (n = 3) and 3.7 % (n = 1) for the BoNT/A and placebo groups, respectively. Further, nine patients were actively withdrawn from the study (BoNT/A groups (n = 5; 8.2 %) and placebo groups (n = 4; 14.8 %)) because they showed no significant treatment effect. The other 10 patients in the anxiety and depression evaluation tests did not have a complete follow-up due to the COVID-19 pandemic or personal reasons. The injectors and evaluators could not predict whether or not their patients were in the BoNT/A group, according to the systems after treatment.

3.3. *Adverse effects*

Common adverse effects of the treatments are summarized in Table 2. After the injection with BoNT/A, the patients were observed for half an hour before they left hospital, and there were no acute side effects observed. Skin tightness, eyebrow asymmetry, and eyelid ptosis occurred in 4.9 %, 3.2 %, and 3.2 %, respectively, in the BoNT/A group, whereas none of these was observed in the placebo group. This phenomenon suggests that the BoNT/A group experienced some side effects due to the local injections. The observed side effects were consistent with those reported in previously published trials (Brin et al., 2020; Wollmer et al., 2012). Furthermore, systemic adverse events of the treatment, such as gastrointestinal effects, were not observed in this study.

4. Discussion

Following a placebo, double-blind randomized controlled study, BoNT/A demonstrated statistically significant antidepressant superiority over placebo. Although the placebo group also had decreased

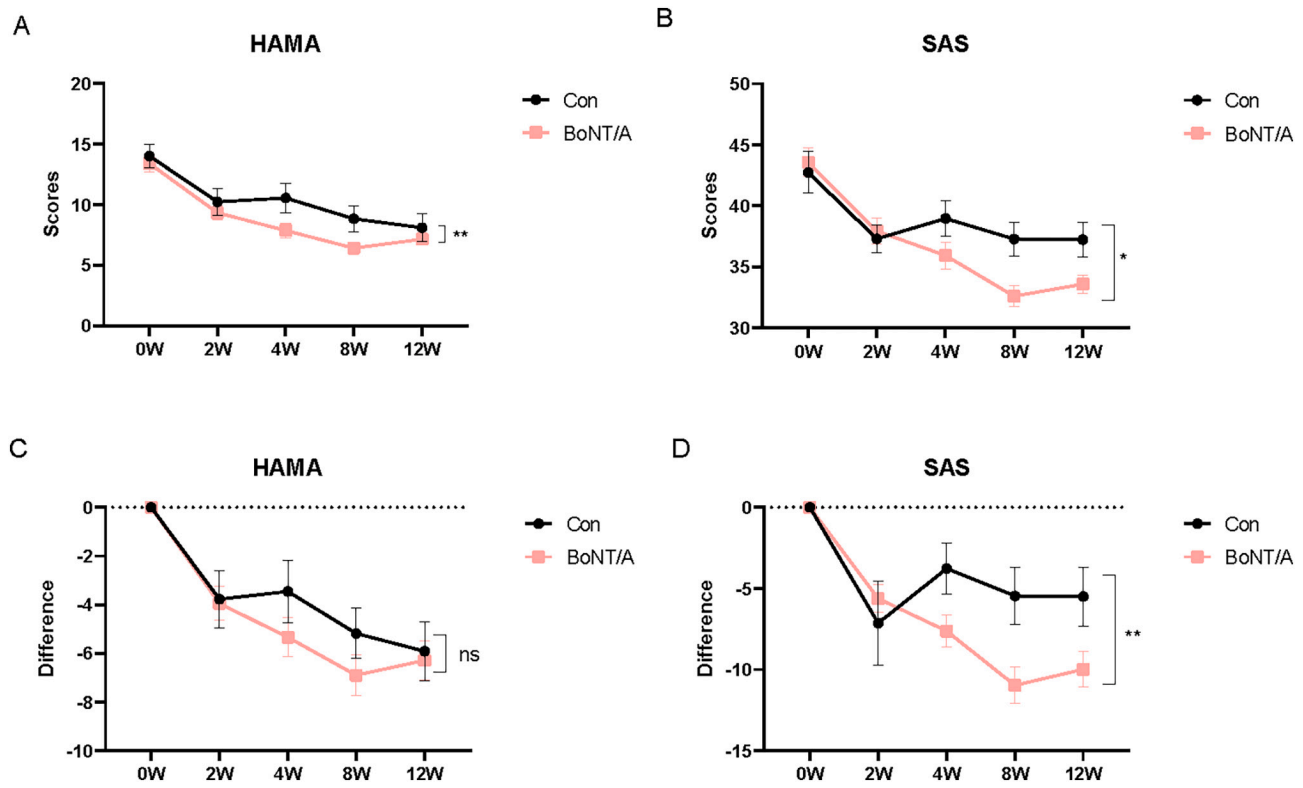

Fig. 4. Changes in HAMA and SAS scores for 100 U treatments.
The changes in the HAMA and SAS scores between the BoNT/A and control groups are shown in A and B. The changes in the HAMA and SAS scores from baseline between the BoNT/A and control groups are shown in C and D. The difference between the BoNT/A and sertraline groups at each time point was determined using two-way repeated-measured ANOVA. *: there was statistical significance between the control and BoNT/A group, $P < 0.05$. Con, control, n = 22; BoNT/A, botulinum toxin A, n = 56; HAMA, Hamilton Depression Scale; SAS, Self-Rating Anxiety Scale; W, week. Data are summarized as mean ± SEM.

Table 2
Summary of adverse events occurring in BoNT/A group.

Adverse events	Control group (N = 27)	BoNT/A group (N = 61)
Skin tightness	0	3 (4.9 %)
Eyebrow asymmetry	0	2 (3.2 %)
Eyelid ptosis	0	2 (3.2 %)
Overall; n (%)	0	7 (11.5 %)

depressive scores for HAMD and HAMA, it was not <7 at the end of the observation period. The observed antidepressant efficacy of BoNT/A was consistent with that of previous trials (Brin et al., 2009; Finzi and Rosenthal, 2014; Wollmer et al., 2012). The efficacy of BoNT/A was evidently more significant in depression than in anxiety as observed from the tested scales. The SDS and SAS scores indicated that the patients also had individualized personal mood experiences after treatment. In addition, the results suggested that BoNT/A had a superior antidepressant efficacy but only moderate anti-anxiety efficacy.

Notably, the injection sites were modified according to the reports of previous studies. The sites were the lateral canthus of the eyes and the bilateral temporal region, and there were some short planks of injections. However, the group numbers were small, and recruiting more patients would have been desirable. Brin et al. conducted a similar trial, where 30 U and 50 U were administered to the BoNT/A group (Brin et al., 2020); however, the present study used 100 U. After a single treatment session, neither BoNT/A 30 U nor 50 U showed statistically significant superiority over placebo. Further studies should be conducted to achieve optimum results with the lowest dose of BoNT/A. This study was conducted in a Chinese general hospital, while several previous studies were carried out in non-Chinese countries. In this study, the evaluators included several persons, who were trained uniformly, thereby making the assessments more objective.

The anti-depressive mechanism of BoNT/A remains unelucidated. However, there are two main hypotheses regarding its mode of action: facial feedback theory and direct changes in neurotransmitter levels in brain regions. The facial feedback theory proposes that facial expressions can affect emotional perceptions, and emotional facial expression is associated with the emotional state of the patient (Nakatani and Yamaguchi, 2014). Muscle activity that controls mood affects neurotransmitter changes in the central nervous system (Heller et al., 2014; Kim et al., 2014; Lee et al., 2012).

In facial feedback theory, BoNT/A indirectly alters the emotion-related transmitters. The explanation is that BoNT/A blocks the cholinergic synapse between the lateral branches of motor neurons (Marchand-Pauvert et al., 2013). It also inhibits the release of acetylcholine at the neuromuscular junction to induce temporary denervation of the affected muscle, which finally leads to typical flaccid paralysis in the injected muscle (Heckmann et al., 2003). Therefore, injecting BoNT/A into the muscles decreases the expression of negative moods. Results of a previous study showed that the activity of the amygdala was decreased when patients expressed anger after BoNT/A injections in the frowning muscle by functional magnetic resonance imaging (Hennenlotter et al., 2009). The findings of our previous study highlighted that BoNT/A could significantly improve the depressive performance of mice in the forcing swimming test and tail suspension test (Li et al., 2019).

The antidepressant effects of BoNT/A are associated with enhanced expression levels of 5-hydroxytryptamine (5-HT) and BDNF in mice brains and activated BDNF/ERK/CREB pathways (Li et al., 2019). The results suggest that BoNT/A may directly change the levels of emotion-related transmitters. Some research findings also showed that BoNT/A can be reverse transported to the brain or advanced neuron, where it exerts the effect (Caleo and Restani, 2018; Mazzocchio and Caleo, 2015;

Restani et al., 2011). Previous studies reported that injections of BoNT/A reduce tetanus toxin-evoked spastic paralysis (Matak, 2020). Elsewhere, the beneficial effects of BoNT/A and the occurrence of cleaved SNAP-25 in the ventral horn were prevented by the antitoxin (Matak, 2020). This provides evidence for the central effect of BoNT/A and lends support to the premise that BoNT/A plays a direct role in the brain. Furthermore, retrograde transportation of BoNT/A1 can arrive at the spinal cord after a subcutaneous or intramuscular injection (Koizumi et al., 2014). These results suggest that BoNT/A may utilize an active retro-axonal transport and directly play a role in brain regions.

The limitations of the study include the absence of a multicenter study and an inadequate number of cases. Additionally, the mechanism of BoNT/A antidepression was not studied.

This study showed the safety and efficacy of BoNT/A as an antidepressant. Botulinum toxin A therapy was found to be fundamentally different from most established psychiatric treatment approaches. Hence, this therapy may provide novel targets for developing effective antidepressant drugs. The study also revealed the necessity for exploring the neurobiological correlation and mechanisms of the mood-lifting effect of BoNT/A.

CRediT authorship contribution statement

YL, TZ, and TT Shen tested the scales and prepared the draft. WQ Wu, JQ Cao, JW Sun, XP Zhou, CX Jiang, and ZT collected some data. TL, LC, HH, and WF Luo contributed to the work design. HH and WF Luo wrote the manuscript. All authors contributed to the writing and approved the submitted version of the article.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgments

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