RESEARCH ARTICLE

Potential Disease-Modifying Effects of Ganglioside GM1 Pulse Treatment on Spinocerebellar Ataxia Type 3, a Parallel-Group, Double-Blind, Randomized, Controlled Trial

Yong-Kang Chen, MD,[1,2] Hai-Yan Tian, MD,[1] Qing-Yong Zhu, MD,[1] Rui Zhang, MD,[1,2] Dong-Xiao Liang, MD,[1,2] Jiu-Qi Wang, MM,[1,2,3] Ren-Yi Feng, MM,[1] Chi Qin, MM,[1] Ming-Ming Ma, MD, PhD,[4] Hong Jiang, MD, PhD,[5] Bei-Sha Tang, MD, PhD,[6,7] Xue-Bing Ding, MD, PhD,[1,2] and Xue-Jing Wang, MD, PhD[1,2,6,7*]

[1]*Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China*
[2]*Henan Key Laboratory of Chronic Disease Prevention and Therapy & Intelligent Health Management, Zhengzhou, China*
[3]*Academy of Medical Sciences of Zhengzhou University, Zhengzhou, China*
[4]*Department of Neurology, Affiliated People's Hospital of Zhengzhou University, Henan Provincial People's Hospital, Zhengzhou, China*
[5]*The Third Xiangya Hospital of Central South University, Central South University, Changsha, China*
[6]*Department of Neurology, Multi-Omics Research Center for Brain Disorders, The First Affiliated Hospital, University of South China, Hengyang, China*
[7]*Clinical Research Center for Immune-Related Encephalopathy of Hunan Province, Hengyang, China*

ABSTRACT: Background: Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant inherited neurodegenerative disorder for which there is currently no cure, nor effective treatment strategy.
Objective: Our aim was to investigate the safety and efficacy of high-dose ganglioside GM1 (ganglioside-monosialic acid) pulse treatment in patients with SCA3.
Methods: Patients were randomly allocated to receive either high-dose GM1 (400 mg on the first day followed by 200 mg/day), low-dose GM1 (40 mg/day), or placebo (normal saline) for 4 weeks. The primary outcome, assessed by measuring the change in the Scale for the Assessment and Rating of Ataxia (SARA) scores from baseline to 12 weeks post-treatment, is central to evaluating treatment efficacy. Secondary outcomes included changes in the International Cooperative Ataxia Rating Scale (ICARS) score, Barthel Index of Activities of Daily Living (ADL), and plasma and cerebrospinal fluid (CSF) GABA levels. Safety was assessed in all treated patients.
Results: A total of 48 patients with SCA3 were enrolled in this study. After 12 weeks, data from 43 patients were included in the efficacy analysis (intention-to-treat analysis). The least-squares mean change in the SARA score from baseline to 12 weeks post-treatment was −3.80 (standard error [SE], 0.39; 95% confidence interval [CI], −4.58 to −3.02) in the high-dose GM1 group, 0.34 (SE, 0.40; 95% CI, −0.46 to 1.13) in the low-dose GM1 group, and 0.73 (SE, 0.40; 95% CI, −0.07 to 1.52) in the placebo group, respectively. Secondary outcomes showed improvements in the ICARS score, Barthel Index of ADL, and plasma and CSF GABA levels in the high-dose GM1 group compared to the low-dose GM1 and placebo groups. All treatments were well-tolerated and safe.
Conclusions: High-dose GM1 treatment significantly ameliorated ataxic symptoms in patients with SCA3. © 2024 International Parkinson and Movement Disorder Society.

Key Words: ganglioside GM1; SARA; SCA3

*__Correspondence to:__ Prof. X.J. Wang, Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; E-mail: fccwangxj2@zzu.edu.cn

Relevant conflicts of interest/financial disclosures: The authors declare that there is no conflict of interest.

Funding agency: The National Natural Science Foundation of China (82122022, 82471272, 82471350, and 82171248), Natural Science Foundation of Henan Province for Distinguished Young Scholars (222300420017), Henan Scientific and Technological Development Program (232301420015), Funding for Scientific Research and Innovation Team of The First Affiliated Hospital of Zhengzhou University (ZYCXTD2023003 and QNCXTD2023008), and Clinical Medicine First-Class Discipline Talent Cultivation Program of Zhengzhou University (32410700TP010).

Received: 6 June 2024; **Revised:** 30 September 2024; **Accepted:** 14 October 2024

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.30050

Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD), is an autosomal dominantly inherited neurodegenerative disease caused by the abnormal expansion of CAG repeats in the *ATXN3* gene,[1] which characterized by progressive ataxia, dystonia, spasticity, and various other symptoms.[2,3] Currently, the therapeutic strategies primarily focus on symptom management.[4,5] Over the past few decades, several pharmacological treatments have been evaluated in clinical trials for their efficacy against SCAs, such as riluzole,[6] rovatirelin,[7] valproic acid,[8] varenicline,[9] and lithium carbonate.[10] However, to date, none of these therapies have shown significant clinical benefits. Additionally, non-pharmacologic approaches, including rehabilitative therapy,[11] non-invasive neurostimulation,[12,13] and branched-chain amino acids,[14] as well as disease modifying therapies such as CRISPR/Cas9[15] and antisense oligonucleotides,[16] have shown therapeutic potential. Although these findings are promising, further research is necessary to confirm their efficacy and safety in SCA patients.

Cerebellar Purkinje neurons (PNs) are large GABAergic projection neurons that carry the sole output of the cerebellar cortex,[17] endowed with elaborate dendrites that receive numerous excitatory inputs.[18] A preclinical study has shown that reduced excitability of PNs contributes to motor dysfunction in ataxia patients.[19] In ataxia patients, glutamic acid decarboxylase (GAD) autoantibodies in cerebrospinal fluid (CSF) act on the nerve terminals of GABAergic neurons, leading to decreased GABA release.[20] Therefore, enhancing the excitability of PNs and increasing GABA levels could serve as potential therapeutic target for partially alleviating ataxic symptoms.

Ganglioside-monosialic acid (GM1) is the predominant ganglioside in the vertebrate brain,[21,22] exerting neurotrophic and neuroprotective effects such as neurogenesis, nerve development, and differentiation.[23-25] To date, GM1 has been shown therapeutic potential for the prevention and treatment of neurodegenerative diseases, including Huntington's disease (HD), Parkinson's disease (PD), and Alzheimer's disease.[26] In HD mouse models, GM1 treatment was found to reduce mutant huntingtin protein levels, restore neurotransmitters levels, and improve motor performances.[27] This clinical trial assessed the effectiveness and safety of GM1 pulse treatment in SCA3 patients, indicating its potential as a disease-modifying therapy for SCA3.

Methods

Study Design

This trial was a 16-week, monocenter, randomized, parallel-group, placebo-controlled trial, conducted between September 2022 and April 2023. Each patient received a 4-week treatment and a 12-week follow-up. This study was approved by the Research and Clinical Trial Ethics Committee of the First Affiliated Hospital of Zhengzhou University (2021-KY-1015) and registered with the Chinese Clinical Trial Registry (registration number: ChiCTR2200063867). The recruitment was conducted from September 2022 to January 2023. The treatment was conducted in February 2023 with a 12-week follow-up until May 2023. The full trial protocol can be accessed in the Supplementary Protocol File.

Eligibility and Exclusion Criteria

The inclusion criteria for SCA3 were as follows: (1) subjects with the symptom and sign of ataxia, age ≥18 years; (2) subjects genetically diagnosed with SCA3/MJD, or whose pedigree gene has been identified with SCA3/MJD; (3) subjects having the ability to understand and provide written informed consent and voluntarily consent to participate in the study. The exclusion criteria were as follows: (1) patients with hereditary ataxia identified through recessive inheritance, X-linked, and mitochondria; (2) patients having been excluded with SCA3/MJD by genetic diagnosis; (3) patients who were infected with coronavirus 2019 (COVID-19) before the treatment. All diagnosis of SCA3 was independently carried out by two neurologists and two geneticists with more than 5 years of clinical experience.

Sample Size Estimation, Randomization, and Blinding

To determine the sample size needed for comparing the efficacy of high-dose GM1 and low-dose GM1 against the placebo group, the formula is as follows:

$$n = \frac{2\left(Z_{1-2/\alpha} + Z_{1-\beta}\right)^2 \sigma^2}{\delta^2}$$

Where n is the estimated sample size; α is the type I error rate; β is the type II error rate; σ is the population standard deviation (SD); and δ is the allowable error. Based on an assumed 20% dropout rate, a total of 60 participants (20 per arm) will provide 80% power to detect a 5-point reduction of the Scale for the Assessment and Rating of Ataxia (SARA) score (the assumed SD is 5 based on our preclinical investigation) in high-dose GM1 group or low-dose GM1 group compared to placebo group at $\alpha = 0.05$. Stratification was conducted based on two factors: age and CAG repeat numbers (<65 and ≥65). Each stratum was further divided into blocks, with each containing a fixed number of participants. Within each block, participants were randomly assigned to one of the three treatment groups in a 1:1:1 ratio. The randomization was performed

by an independent statistician who was blinded to the treatment or clinical decisions affecting the patients. The SCA3 patients were randomly divided into three groups: the high-dose GM1 group, where patients received 400 mg of GM1 intravenously on the first day and followed by 200 mg/day for 4 weeks; the low-dose GM1 group, where patients received 40 mg/day of continuous intravenous GM1 for 4 weeks; and the placebo group, where patients received an equal volume of continuous intravenous normal saline (NS) for 4 weeks, administered each day between 8:00 to 10:00 am. All infusions were carried out in the hospital. Both patients and investigators were blinded to randomization process.

Outcome Measurements

The primary outcome measure is the least-squares mean (LSM) change of the SARA score between baseline and 12 weeks post-treatment (week 16). The SARA score ranges from 0 to 40, with higher scores indicating more severe ataxia,[28] including 8 items: gait, stance, sitting, speech disturbance, finger chase, nose-finger test, fast alternating hand movements, and heel-shin slide.[29] The main secondary outcomes were the progression of cerebellar motor deficits between baseline and the end of treatment (week 4), 6 weeks post-treatment (week 10), or week 16, as measured by the International Cooperative Ataxia Rating Scale (ICARS) score.[30] The ICARS score ranges from 0 to 100, with higher scores indicating more severe impairment. Additional secondary outcomes between baseline and weeks 4, 10, or 16 were physical disability as assessed by the Barthel Index of Activities of Daily Living (ADL) (range from 0 to 100, with higher scores indicating less disability),[31] plasma and CSF GABA levels. Plasma and CSF samples were collected between 8:00 and 10:00 am. The blood samples were derived from fasting venous blood collection, whereas the CSF samples were obtained through lumbar puncture. All investigators were experienced in using these rating scales. In addition, adverse events (AEs) were assessed throughout the treatment.

Patient Data Collection and Follow-Up

In addition to age, sex, CAG repeats, and disease duration, the neurological functions of each participant, such as muscle spasm, hyperreflexia, dysarthria, dysphagia, and sensory loss, were examined at baseline. Patient follow-ups were conducted through direct patient contact. The severity of ataxia symptoms after GM1 treatment discontinuation was assessed using SARA and ICARS scores. Scoring was conducted by a single blinded observer to minimize error in evaluating SCA3 patients. Besides SARA and ICARS scores, the Barthel Index of ADL, plasma and CSF GABA levels were also measured at weeks 4, 10, and 16.

Neurotransmitter Detection

Ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) analysis was carried out to detect the GABA levels. CSF samples (10 mL) were collected in polypropylene tubes after overnight fasting and centrifuged at 3000 *g* for 10 minutes at room temperature, whereas blood samples (5 mL) were collected in plastic tubes with potassium-EDTA, centrifuged at 3000 rpm at 4°C for 20 minutes to separate plasma. Next, 0.5 mL of CSF or plasma aliquots were flash-frozen in liquid nitrogen and stored at −80°C until analysis.

Statistical Analysis

Before the analysis, data inspection was conducted by two inspectors in this study to ensure the completeness and accuracy of the input data. Qualitative data are expressed as number of observations with percentage, and quantitative data are expressed as the mean and SD, or median and interquartile ranges (IQRs) analyzed by the independent sample *t* test. Data of two groups were compared using two-sample Student's *t* test if they were normally distributed, otherwise Mann–Whitney test would be used. Statistical analyses were performed using SPSS version 27.0 and GraphPad Prism 9 (GraphPad Software, La Jolla, CA). For all statistical tests, significance was taken as not significant (n.s.), $P \geq 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. The primary and secondary analyses for the baseline and final evaluation data were conducted using linear mixed models for repeated measures. The linear mixed model was used with age, CAG repeats, and age of onset as a random factor, whereas the week, group, and their interaction were a fixed factor. For the comparisons among the three groups, significance was taken as $^{*}P < 0.05$. The Bonferroni correction was applied to create a significance threshold corrected for multiple testing (Bonferroni threshold: $0.05/3 = 0.0167$), and $^{\#}P < 0.0167$ was considered statistically different after Bonferroni correction.

Results

Participants

A total of 80 subjects with genetically confirmed SCA3 were screened, and 48 subjects, including 22 males and 26 females, were randomly assigned to either the low-dose GM1 group (n = 16), high-dose GM1 group (n = 16), or the placebo group (n = 16). During the follow-up period, a total of five patients were lost to follow-up because of COVID-19 infection: two from the placebo group, two from the low-dose GM1 group, and one from the high-dose GM1 group. Overall, 43 subjects completed the 12-week follow-up from September 2022 to May 2023 (Fig. 1). The mean

age of the 48 patients who complete the 4-week treatment was 42.25 ± 9.28 years (range: 22 to 61), with a mean age of onset of 32.23 ± 8.79 years (range: 16 to 50), a mean disease duration of 10.02 ± 3.13 years (range: 4 to 20), and a mean number of CAG repeats of 64.48 ± 8.48 (range: 47 to 83). Baseline characteristics were shown in Table 1. All patients participating in study assessments were blinded to the treatment assignments throughout the study.

Primary Outcome

The primary efficacy measure was the change in LSM of the SARA score from baseline to week 16. In the intention-to-treat (ITT) analysis, the LSM change from baseline to week 16 was 0.73 (standarad error [SE], 0.40; 95% confidence interval [CI], −0.07 to 1.52) of the placebo group (n = 14), 0.34 (SE, 0.40; 95% CI, −0.46 to 1.13) for the low-dose GM1 group (n = 14), and −3.80 (SE, 0.39; 95% CI, −4.58 to −3.02) in the high-dose GM1 group (n = 15) (Table 2). This represents a treatment benefit of 4.52 (SE, 0.56; 95% CI, 3.42 to 5.63, $P < 0.0001$) for the high-dose GM1 group compared to the placebo group. Furthermore, no significant differences in the total SARA score were observed for either the high-dose GM1 group or low-dose GM1 group compared to the placebo group at week 16. However, significant improvements were detected in the high-dose GM1 group at weeks 4 and 10 (Supplementary Fig. S1 and Supplementary Table S1).

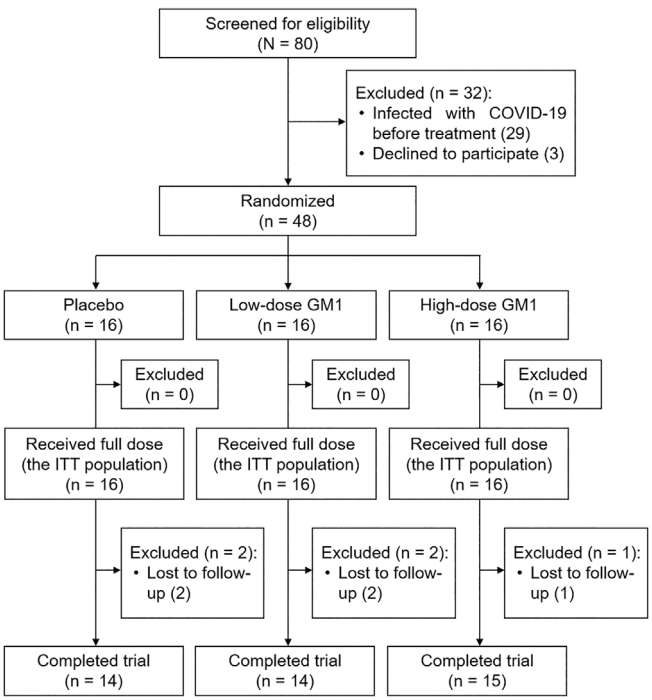

FIG. 1. The enrollment, randomization, and follow-up of SCA3 patients.

Additionally, the high-dose GM1 group showed significant improvements (change from baseline to week 16) in gait (SE, −0.79; 95% CI, −1.05 to −0.53; $P < 0.0001$), stance (SE, −0.79; 95% CI, −0.96 to −0.62; $P < 0.0001$), speech disturbance (SE, −0.45; 95% CI, −0.60 to −0.30; $P < 0.0001$), finger chase (SE, −0.46; 95% CI, −0.62 to −0.30; $P = 0.0004$), and fast alternating movements (SE, −0.72; 95% CI, −0.87 to −0.58; $P < 0.0001$) compared to the placebo group. Conversely, the low-dose GM1 group did not exhibit significant differences in any of the SARA subscores (Table 2). Moreover, reductions in SARA subscores, including those for gait, stance, and fast alternating hand movements, were observed at weeks 4, 10, and 16 in the high-dose GM1 group compared to the placebo group. No significant differences were found between the low-dose GM1 and the placebo groups (Supplementary Fig. S1 and Supplementary Table S1).

Secondary Outcomes

ICARS Score

As shown in Table 2, the high-dose GM1 group showed significantly decreased ICARS score from baseline to week 4 (SE, −11.55; 95% CI, −13.05 to −10.04; $P < 0.0001$), week 10 (SE, −10.64; 95% CI, −12.17 to −9.11; $P < 0.0001$), and week 16 (SE, −8.90; 95% CI, −10.43 to −7.38; $P < 0.0001$) compared to the placebo group, respectively. Conversely, no significant differences were observed in the low-dose GM1 group when compared to the placebo group. Additionally, as shown in Fig. 2A and Supplementary Table S1, the ICARS score demonstrated significant reductions in the high-dose GM1 group compared to the placebo group at weeks 4, 10, and 16, respectively. However, no statistically significant differences were observed between the low-dose GM1 group and the placebo group.

Barthel Index of ADL

Significant improvements were observed in the Barthel Index of ADL following high-dose GM1 treatment (Table 2). The LSM values in the Barthel Index of ADL from baseline to weeks 4, 10, and 16 in the high-dose GM1 group were significantly higher than those in the placebo group (week 4: SE, 12.19; 95% CI, 9.97–14.41; $P < 0.0001$; week 10: SE, 10.67; 95% CI, 8.40 to 12.93; $P < 0.0001$; week 16: SE, 8.67; 95% CI, 6.40–10.93; $P < 0.0001$). No significant differences were noted in the low-dose GM1 group compared to the placebo group (Table 2). Additionally, the improved Barthel Index of ADL was found in the high-dose GM1 group compared to the placebo group at week 4 (Fig. 2B and Supplementary Table S1).

TABLE 1 *The demographics and baseline characteristics of SCA3 patients in this study*

	Placebo (n = 16)	Low-dose GM1 (n = 16)	High-dose GM1 (n = 16)
Age, mean (SD), y	42.2 (7.1)	42.3 (10.7)	42.3 (10.3)
Race, Asian, n (%)	16 (100)	16 (100)	16 (100)
Sex, female, n (%)	7 (44)	8 (50)	7 (44)
BMI, mean (SD), kg/m^2	18.15 (1.54)	17.99 (1.49)	17.47 (1.41)
CAG repeats, mean (SD)	63.88 (8.24)	65.38 (8.22)	64.19 (9.41)
Age of onset, mean (SD), y	31.69 (7.80)	32.13 (9.65)	32.88 (9.34)
Disease duration, mean (SD), y	10.50 (4.16)	10.13 (2.13)	9.44 (2.85)
Dystonic postures, n (%)	7 (44)	7 (44)	6 (38)
Hyperreflexia, n (%)	9 (56)	8 (50)	9 (56)
Dysarthria, n (%)	7 (44)	7 (44)	5 (31)
Dysphagia, n (%)	5 (31)	6 (38)	7 (44)
Superficial or deep sensory loss, n (%)	10 (63)	8 (50)	9 (56)
SARA score			
Total, mean (SD)	17.88 (4.54)	17.44 (3.74)	17.56 (5.39)
Gait, mean (SD)	4.25 (1.29)	4.13 (0.96)	4.00 (1.27)
Stance, mean (SD)	2.88 (0.72)	2.94 (0.44)	2.88 (0.81)
Sitting, mean (SD)	0.88 (0.34)	0.88 (0.34)	0.94 (0.68)
Speech disturbance, mean (SD)	2.13 (0.34)	2.19 (0.40)	2.31 (0.48)
Finger chase, mean (SD)	1.69 (0.48)	1.69 (0.48)	1.69 (0.60)
Nose-finger test, mean (SD)	1.25 (0.50)	1.25 (0.45)	1.19 (0.75)
Fast alternating hand movements, mean (SD)	2.63 (0.49)	2.44 (0.51)	2.50 (0.52)
Heel-shin slide, mean (SD)	2.19 (0.83)	1.94 (1.00)	2.06 (0.93)
ICARS score, mean (SD)	37.88 (9.33)	36.81 (7.31)	37.13 (10.68)
Barthel Index, mean (SD)	63.75 (13.96)	63.13 (11.53)	65.31 (13.72)
Plasma GABA levels, mean (SD), ng/mL	14.10 (4.12)	13.68 (4.40)	13.94 (4.13)
CSF GABA levels, mean (SD), ng/mL	15.69 (4.90)	14.58 (4.71)	15.19 (3.66)

Abbreviations: SCA3, spinocerebellar ataxia type 3; GM1, ganglioside-monosialic acid; SD, standard deviation; BMI, body mass index; SARA, Scale for the Assessment and Rating of Ataxia; ICARS, International Cooperative Ataxia Rating Scale; CSF, cerebrospinal fluid.

Plasma and CSF GABA Levels

Prior to GM1 treatment, chromatographic separation was carried out to analyze plasma and CSF GABA levels in both SCA3 patients and healthy controls (HCs). The results revealed decreased GABA levels in both plasma and CSF samples from SCA3 patients when compared to HCs (Fig. 2C,D). Following GM1 treatment, significant changes in plasma GABA levels were observed from baseline to weeks 4, 10, and 16 in the high-dose GM1 group compared to the placebo group. Specifically, the high-dose GM1 group showed increased plasma GABA levels at week 4 (SE, 2.50; 95% CI, 1.75–3.24; $P < 0.0001$), week 10 (SE, 1.80; 95% CI, 1.04–2.55; $P = 0.0011$), and week 16 (SE, 1.40; 95% CI, 0.64–2.16; $P = 0.0094$). Conversely, the low-dose GM1 group showed no significant changes in plasma GABA levels at these time points (Table 2). Additionally, significant differences were observed in the changes of CSF GABA level from baseline to weeks 4, 10, and 16 in the high-dose GM1 group compared to the placebo group (week 4: SE, 3.07; 95% CI, 2.03–4.11, $P < 0.0001$; week 10: SE, 2.87; 95% CI, 1.81–3.93; $P < 0.0001$; week 16: SE, 2.61; 95% CI, 1.56–3.67; $P < 0.0001$), respectively. In addition, the low-dose GM1 group displayed comparable changes in CSF GABA level to the placebo group at these time points (Table 2). Furthermore, no significant differences in plasma and CSF GABA levels were detected between

TABLE 2 *The primary and secondary outcomes in this study*

Outcome	Placebo	Low-dose GM1	High-dose GM1	P_1 value	P_2 value	P_3 value
SARA score, total						
Change from baseline to week 16, LSM (95% CI)	0.73 (−0.07 to 1.52)	0.34 (−0.46 to 1.13)	−3.80 (−4.58 to −3.02)	<0.0001*	0.7712	<0.0001**
SARA subscore, change from baseline to week 16						
Gait, LSM (95% CI)	0.23 (−0.04 to 0.50)	0.23 (−0.05 to 0.48)	−0.79 (−1.05 to −0.53)	<0.0001*	0.9953	<0.0001**
Stance, LSM (95% CI)	0.21 (0.03–0.38)	0.01 (−0.17 to 0.18)	−0.79 (−0.96 to −0.62)	<0.0001*	0.2635	<0.0001**
Sitting, LSM (95% CI)	0.01 (−0.11 to 0.12)	0.00 (−0.11 to 0.11)	−0.14 (−0.24 to −0.03)	0.0754	0.9984	0.1758
Speech disturbance, LSM (95% CI)	0.14 (−0.02 to 0.30)	−0.01 (−0.16 to 0.15)	−0.45 (−0.60 to −0.30)	<0.0001*	0.4053	<0.0001**
Finger chase, LSM (95% CI)	0.01 (−0.16 to 0.17)	−0.07 (−0.23 to 0.10)	−0.46 (−0.62 to −0.30)	0.0001*	0.8126	0.0004**
Nose-finger test, LSM (95% CI)	0.08 (−0.07 to 0.22)	0.01 (−0.14 to 0.16)	−0.14 (−0.28 to 0.01)	0.0437*	0.8244	0.1076
Fast alternating hand movements, LSM (95% CI)	0.00 (−0.15 to 0.15)	0.08 (−0.07 to 0.23)	−0.72 (−0.87 to −0.58)	<0.0001*	0.7331	<0.0001**
Heel-shin slide, LSM (95% CI)	0.07 (−0.15 to 0.29)	0.09 (−0.13 to 0.31)	−0.33 (−0.54 to −0.12)	0.0122*	0.9886	0.0323
ICARS score						
Change from baseline to week 4, LSM (95% CI)	0.35 (−1.15 to 1.85)	−0.16 (−1.66 to 1.33)	−11.55 (−13.05 to −10.04)	<0.0001*	0.8804	<0.0001**
Change from baseline to week 10, LSM (95% CI)	0.82 (−0.73 to 2.38)	0.51 (−1.05 to 2.06)	−10.64 (−12.17 to −9.11)	<0.0001*	0.9566	<0.0001**
Change from baseline to week 16, LSM (95% CI)	1.04 (−0.52 to 2.59)	1.01 (−0.55 to 2.56)	−8.90 (−10.43 to −7.38)	<0.0001*	0.9996	<0.0001**
Barthel Index of ADL						
Change from baseline to week 4, LSM (95% CI)	−0.98 (−3.20 to 1.23)	0.16 (−2.06 to 2.37)	12.19 (9.97 to 14.41)	<0.0001*	0.7508	<0.0001**
Change from baseline to week 10, LSM (95% CI)	−1.44 (−3.75 to 0.87)	−1.27 (−3.57 to 1.04)	10.67 (8.40 to 12.93)	<0.0001*	0.9939	<0.0001**
Change from baseline to week 16, LSM (95% CI)	−1.80 (−4.11 to 0.51)	−1.63 (−3.93 to 0.68)	8.67 (6.40 to 10.93)	<0.0001*	0.9939	<0.0001**
Plasma GABA levels, ng/mL						
Change from baseline to week 4, LSM (95% CI)	−0.63 (−1.37 to 0.11)	−0.07 (−0.81 to 0.67)	2.50 (1.75 to 3.24)	<0.0001*	0.5368	<0.0001**
Change from baseline to week 10, LSM (95% CI)	−0.23 (−1.01 to 0.55)	−0.36 (−1.14 to 0.41)	1.80 (1.04 to 2.55)	0.0004*	0.9685	0.0011**
Change from baseline to week 16, LSM (95% CI)	−0.25 (−1.03 to 0.53)	−0.90 (−1.67 to −0.12)	1.40 (0.64 to 2.16)	0.0034*	0.4767	0.0094**
CSF GABA levels, ng/mL						
Change from baseline to week 4, LSM (95% CI)	−0.74 (−1.77 to 0.30)	−0.55 (−1.59 to 0.48)	3.07 (2.03 to 4.11)	<0.0001*	0.9673	<0.0001**
Change from baseline to week 10, LSM (95% CI)	−0.71 (−1.79 to 0.37)	−0.58 (−1.66 to 0.50)	2.87 (1.81 to 3.93)	<0.0001*	0.9848	<0.0001**
Change from baseline to week 16, LSM (95% CI)	−1.35 (−2.43 to −0.27)	−0.87 (−1.95 to 0.21)	2.61 (1.56 to 3.67)	<0.0001*	0.8087	<0.0001**

The P_1 values are comparisons among the three groups, $*P < 0.05$ was considered statistically different. The P_2 and P_3 values are comparisons between the low-dose GM1 or the high-dose GM1 group and the placebo group, respectively, $**P < 0.0167$ was considered statistically different after Bonferroni correction.

Abbreviations: GM1, ganglioside-monosialic acid; SARA, Scale for the Assessment and Rating of Ataxia; LSM, least-squares mean; CI, confidence interval; ICARS, International Cooperative Ataxia Rating Scale; ADL, activities of daily living.

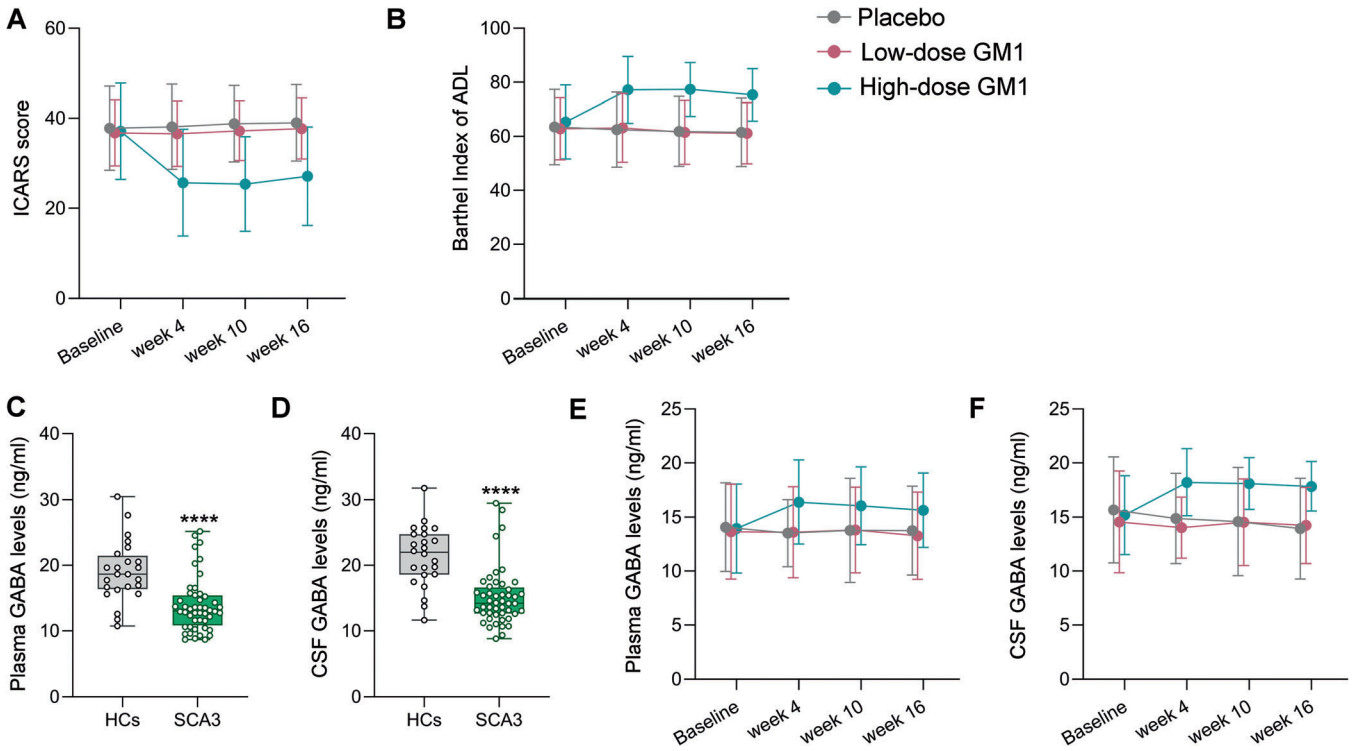

FIG. 2. (**A**,**B**) The International Cooperative Ataxia Rating Scale (ICARS) score (**A**) and Barthel Index of Activities of Daily Living (ADL) (**B**) at baseline, weeks 4, 10, and 16, respectively. (**C**,**D**) The comparisons of plasma (**C**) and cerebrospinal fluid (CSF) (**D**) GABA levels in healthy controls (HCs) (n = 24) and spinocerebellar ataxia type 3 (SCA3) patients (n = 48) at baseline. ****$P < 0.0001$. The P values are comparisons to the HCs group. (**E**,**F**) The plasma (**E**) and CSF (**F**) GABA levels at baseline, weeks 4, 10, and 16, respectively. Data are presented as means ± standard deviation (error bars). [Color figure can be viewed at wileyonlinelibrary.com]

the high-dose GM1 group (or the low-dose GM1 group) and the placebo group at weeks 4, 10, and 16, as illustrated in Fig. 2E,F and Supplementary Table S1. In summary, these findings suggest that high-dose GM1 treatment may function by restoring GABA levels.

Sex Differences and Treatment Effect Analyses

To investigate potential differences in the efficacy of GM1 treatment between male and female patients with SCA3, a sex difference analysis was conducted. As shown in Supplementary Table S2, no significant sex differences were observed in the SARA total score, ICARS score, Barthel Index of ADL, or plasma and CSF GABA levels from baseline to week 16 in SCA3 patients.

Additionally, models including interactions between time (week) and group were compared to models without interactions. This comparison revealed a statistically significant difference ($P < 0.0001$), with the interaction model demonstrating lower Akaike information criterion and Bayesian information criterion values, indicating an interaction between time and group.

Safety and Tolerability

During the 16-week trial period, the most commonly reported AEs were injection site reactions, such as irritation (7/48, 14.6%), hardness (4/48, 8.3%), pain (4/48, 8.3%), and erythema (2/48, 4.2%) (Table 3). All injection site reactions were mild and transient, resolving spontaneously without medical intervention. Other AEs included transient dermatologic reactions (4/48, 8.3%), nausea (2/48, 4.2%), fatigue (2/48, 4.2%), myalgia-arthralgia (1/48, 2.1%), diarrhea (2/48, 4.2%), rash (1/48, 2.1%), and insomnia (1/48, 2.1%) (Table 3). Importantly, no death or serious AEs were reported, and none of the observed AEs were causally linked to the study drug. These findings suggest that high-dose pulse GM1 treatment was well tolerated in SCA3 patients.

Discussion

Previous studies have shown that SCA3 is the most common subtype of SCAs in mainland China, accounting for ~63% of all cases, followed by Brazil, Japan, and Germany.[32] Gait ataxia is the primary symptom of

TABLE 3 *The adverse events reported in this study*

Adverse events	No. of patients		
	Placebo (n = 16)	Low-dose GM1 (n = 16)	High-dose GM1 (n = 16)
No. of patients with at least one adverse event	7	7	6
No. of adverse events per patient	1 or 2	1 or 2	1 or 2
Nausea	1	0	1
Fatigue	1	1	0
Transient dermatologic reactions	2	1	1
Injection site reactions			
Irritation	2	3	2
Hardness	2	1	1
Pain	1	2	1
Erythema	1	0	1
Myalgia-arthralgia	0	1	0
Diarrhea	1	0	1
Rash	0	1	0
Insomnia	0	1	0
Increased antibody titers for GM1 ganglioside	0	0	0

Abbreviation: GM1, ganglioside-monosialic acid.

SCA3, with an average survival duration of 20 to 25 years after symptom onset.[33,34] Despite advancements in understanding the genetic cause of SCA3, effective treatments to slow its progression remain elusive.[35,36] Currently, potential treatments for SCAs include pharmacological and non-pharmacological interventions, gene therapy, neurostimulation, and molecular targeting.[37] Our study demonstrates a significant improvement in clinical symptoms among SCA3 patients receiving high-dose GM1 treatment. Following a 4-week regimen of high-dose pulse GM1, ataxic symptoms in SCA3 patients showed remarkable improvement, persisting for at least 12 weeks, which suggests potential disease-modifying effects. In contrast, low-dose GM1 treatment did not yield improvements compared to the placebo group, indicating a possible dose-dependent efficacy of GM1 treatment.

In this study, we used the SARA as the primary outcome measure to assess cerebellar ataxia in SCA3 patients. Changes in SARA scores reflect the patient's perception of disease status.[38] In a natural history study in Europe, the annual increasing rate of SARA score was 1.56 (0.08) in patients with SCA3.[39] Additionally, a decrease of at least 1 to 1.5 points is usually defined as a relevant improvement.[38] Previous clinical studies have shown that treatments such as varenicline, valproic acid, exergaming training, transcranial direct current stimulation, or repetitive transcranial magnetic stimulation (rTMS) can lead to decreases in mean SARA scores of 1 to 3 points,[8,10,40-42] whereas an improvement in the SARA score of 3.5 points was observed in a 31-year-old SCA3 patient who underwent a trial of neuronavigated, repetitive, low-frequency (1 Hz) rTMS targeting the left dentate nucleus.[43] Remarkably, our study demonstrated a significant reduction in the SARA score (5.75 [0.55] points) following a 4-week high-dose GM1 treatment. However, the underlying mechanisms responsible for this effect require further investigation. Additionally, we included both ICARS and SARA to ensure a comprehensive assessment of motor function and ataxia severity in participants. Although SARA is widely recognized and validated for assessing ataxia, ICARS provides a more detailed evaluation of ataxia symptoms. Our rationale for using both scales was to provide a more comprehensive assessment of the intervention's impact on motor function.

Previous research has identified GM1 as a neuroprotective factor with diverse mechanisms,[44] providing significant benefits in various neurological disorders such as PD, HD, and diabetic peripheral neuropathy.[45-48] Interestingly, a previous study indicated that GM1 administration could restore the levels of GABA in HD mouse models.[27] Considering the commonality of polyglutamine pathology in both SCA3 and HD, we postulated that GM1 might affect GABA levels in SCA3 patients. The decision to use ganglioside GM1 pulse treatment was based on its potential to enhance

neuronal repair through initial high-dose administration aimed at symptom reduction, with preliminary studies suggesting superior therapeutic outcomes with this regimen.

Loss of cerebellar PNs is a primary pathological characteristic of SCAs.[49] Cerebellar PNs receive glutamatergic excitatory inputs from the parallel fibers of granule cells and climbing fibers originated from the inferior olive, as well as GABAergic inhibitory inputs from interneurons.[49-51] Irregular firing of PNs is strongly associated with cerebellar ataxia.[52] PNs release GABA, an inhibitory neurotransmitter crucial for regulating neuronal impulses.[53] Impaired PNs can increase the excitability of deep cerebellar nucleus (DCN) neurons, leading to rapid movement disorders.[54,55] Our study observed a significant increase in GABA levels in both plasma and CSF of SCA3 patients following high-dose GM1 treatment, suggesting that CSF GABA could potentially serve as a biomarker for cerebellar dysfunction. However, further research is necessary to validate its specificity and sensitivity. These findings imply that the upregulation of GABA may restore PN function, enhancing inhibitory output to the DCN and alleviating ataxic symptoms in SCA3 patients. We propose that high-dose GM1 treatment could provide therapeutic for SCA3 by modulating GABA levels in both plasma and CSF.

However, our study has several limitations. First, it was conducted at a single center, which restricts the generalizability of our findings. Second, the relatively small sample size may have limited the statistical power for certain observations. This limitation arose primarily because patients with a history of COVID-19 infection were excluded for several reasons: (1) to ensure the validity and reliability of our findings, as prior infection could introduce varying degrees of cross-immunity or post-infection sequelae, potentially obscuring the effects of the studied factors; (2) to maintain cohort homogeneity and focus on patients without the confounding influence of prior infection, enabling a more accurate evaluation of the variables of interest; (3) previous infections could introduce significant variability in clinical presentations and treatment responses, complicating result interpretation. Moreover, the COVID-19 pandemic posed recruitment and follow-up challenges in China, including community lockdowns, hospital restrictions, procurement issues, and infection risks for site personnel, impacting our ability to complete the planned 24-week follow-up and limiting knowledge of the long-term outcomes of GM1 treatment. Further research is needed to determine whether subsequent GM1 treatments or alternative interventions can sustain the initial improvements observed in SCA3 patients. Last, despite the absence of serious AEs related to high-dose GM1, our data may lack sufficient power to detect rare safety outcomes. Despite the relatively small sample size, our analysis confirmed that the data meet the requirements for a linear mixed-effects model analysis (Supplementary Fig. S2).

In summary, our study provides direct evidence supporting high-dose GM1 treatment as a promising disease-modifying approach for SCA3.

Acknowledgments: The authors thank all the staff and participants of this study for their important contributions.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

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Supporting Data

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X.B.D.: 1B
X.J.W.: 1A, 1B, 2A, 3B