

# A Randomized, Double-blind, Placebo-controlled Trial of DL-3-n-butylphthalide in Amyotrophic Lateral Sclerosis Patients

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## Research

**Keywords:** DL-3-n-butylphthalide, amyotrophic lateral sclerosis, treatment, trial, randomized, double-blind

**Posted Date:** December 17th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1142455/v1>

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# Abstract

**Background:** To determine the efficacy and safety of DL-3-n-butylphthalide (NBP) for the treatment of amyotrophic lateral sclerosis (ALS).

**Methods:** A randomized, double-blind, placebo-controlled trial was performed at 19 ALS clinical centers of the Chinese ALS Association. Patients with definite or probable ALS were randomly treated with NBP or placebo for 12 months. The Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score was the primary endpoint and was evaluated every 3 months. Secondary endpoints included survival and tracheotomy incidence, total Medical Research Council (MRC) score, percentage of predicted forced vital capacity (FVC), and clinical global impression scale score assessed using the visual analog scale.

**Results:** Between November 23, 2015 and November 22, 2017, 312 ALS patients were enrolled and randomly allocated to either the NBP group (156 patients) or placebo group (156 patients). Ninety-three patients in the NBP group and 92 patients in the placebo group were included in the primary end point analysis. There was no significant difference in the ALSFRS-R score, total MRC score, or clinical global impression between the two groups after treatment. The NBP group exhibited a mild trend of less decrease in the percentage of predicted FVC between baseline and the 12-month visit than the placebo group (least-squares mean change from baseline  $\pm$  standard error:  $-7.34 \pm 4.28$ , 95% CI  $(-15.24, 0.56)$ ,  $p=0.0335$ ). Adverse events were reported in 56.5% of patients in the placebo group and 68.8% of patients in the NBP group ( $\chi^2=2.99$ ,  $P=0.0838$ ). No serious adverse event related to treatment occurred.

**Conclusion:** we found no evidence that NBP improved the ALSFRS-R score in patients with ALS. The results suggest a mild trend in the percentage of predicted FVC decreased slowly in the NBP treatment group than in the placebo group.

**Trial registration:** A Multi-center, Randomized, Double Blinding, Placebo-Controlled Clinical Trial of DL-3-Butylphthalide in the Treatment of Amyotrophic Lateral Sclerosis, ChiCTR-IPR-15007365, Registered 1 November 2015, <http://www.chictr.org.cn/showproj.aspx?proj=12354>

## Background

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease in which motor neurons degenerate, resulting in progressive limb and bulbar paralysis and eventually leading to patients requiring mechanical ventilatory support and death. Riluzole and Edaravone have been approved by some countries as treatments to improve the survival of patients with ALS. However, their effects are mild, and a pressing need remains for more effective disease-modifying treatments.

DL-3-n-butylphthalide (NBP) is a synthesized drug that is extracted as a pure component from seeds of *Apium graveolens* Linn (Chinese Celery). Previous studies showed that NBP significantly reduces oxidative damage, improves mitochondrial function, reduces neuronal apoptosis, and inhibits

inflammation (1). In SOD1-G93A transgenic mice, oral administration of 60 mg/kg/d NBP immediately after symptom onset significantly prolonged survival, decreased the progression rate of motor deficits, and suppressed body weight reduction. Furthermore, motor neurons were preserved in the anterior horns compared with mice given a vehicle control (2, 3). Some evidence has indicated that NBP has protective effects in ALS mice; therefore, a placebo-controlled clinical trial was conducted to determine whether a similar effect could be observed in ALS patients. The safety of NBP has been demonstrated in previous stroke studies, and NBP was approved by the State Food and Drug Administration of China for clinical use in stroke patients in 2002. A Chinese ALS group consisting of 19 ALS centers conducted a clinical trial to test the hypothesis that NBP could improve the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) scores in ALS patients.

## Methods

A randomized, double-blind, placebo-controlled trial was performed at 19 ALS clinical centers of the Chinese ALS association. All patients provided written informed consent. The ethics committee of Peking Union Medical College Hospital approved the study protocol. The registration number of the clinical trial was ChiCTR-IPR-15007365.

### Participants

Patients with definite or probable ALS according to the revised version of the El Escorial World Federation of Neurology criteria were recruited into the study (4). Included patients displayed onset of progressive weakness within 18 months prior to the study. The age at onset was between 20 and 75 years old. The forced vital capacity (FVC) was more than 70% of the predicted normal value. Patients had not taken Riluzole or Edaravone in the 3 months before recruitment. All patients were capable of understanding the information provided and giving full informed consent.

The exclusion criteria were as follows: electromyography showed motor nerve conduction block and abnormal sensory nerve conduction; computed tomography or magnetic resonance imaging revealed lesions that may explain the patient's clinical presentation; dementia or mental disorder; serious heart, liver, kidney, or other related disease; participation may endanger the patient's life; gastrointestinal disorders or gastrointestinal surgery that may affect gastrointestinal absorption; a history of allergies, especially medication allergies; patients requiring ventilator-assisted breathing or tracheotomy; patients who were breast feeding or pregnant; patients who had received or were currently receiving Riluzole treatment; presentation of bulbar or thoracic symptoms or signs at onset; or patients who had difficulty taking medicine.

### Sample size calculation

The sample size was calculated according to a previously published clinical trial of ALS patients (5). To provide 80% power to detect an adjusted mean difference between groups of 3 points on the ALSFRS-R score ( $30.2 \pm 8.9$  vs  $33.2 \pm 8.0$ ) and considering a dropout rate of 25% at the last visit, with a 1:1

randomization ratio between NBP and placebo group, at a two-sided alpha level of 0.05, we calculated that a sample size of 312 patients would be required approximately (156 in the NBP group and 156 in the placebo group).

## **Treatment group**

Patients eligible for admission were randomized, using SAS statistical analysis to generate A randomized arrangement of 312 subjects receiving treatment (study drug versus control drug) in A 1:1 ratio between group A and group B. That is, list the serial number, the corresponding treatment assignment, and follow this compilation blind.

Patients randomly assigned to groups A or B were treated with butylphthalide soft capsules (100mg/ tablet) or placebo (A placebo with physical characteristics such as appearance, size, color, dosage form, weight, taste and smell as similar as possible to the experimental drug, but without the active ingredients of the experimental drug).NBP group patients were given two soft capsules(200mg) three times a day for 12 months. Placebo group patients were given two placebo soft capsules three times a day for 12 months.

## **Evaluation of functional status**

All patients were evaluated before treatment with NBP or placebo and then followed up for 12 months. A functional evaluation was conducted every 3 months after enrollment and included the ALSFRS-R, survival and tracheotomy incidence, total Medical Research Council (MRC) score, respiratory function, and clinical global impression (CGI) scale reported by the patient.

The ALSFRS-R score was evaluated according to a questionnaire. The maximum ALSFRS-R score was 48. The total MRC score was calculated as the sum of the muscle strength in 24 muscle movements, including neck flexion, neck extension, bilateral shoulder abduction, elbow flexion, elbow extension, wrist flexion, wrist extension, thumb extension, hip flexion, knee flexion, knee extension, ankle dorsiflexion, and ankle plantar flexion. Each muscle movement was evaluated with the MRC scale (0–5); the total MRC score was 120. Respiration function was monitored with an FVC test and was expressed as a percentage of the expected value. The CGI scale was used to assess disease severity and was evaluated by patients using a visual analogue scale ranging from no symptoms to the most serious symptoms (0 to 7 points).

## **Outcomes**

The primary endpoint was the ALSFRS-R score after 12 months of treatment. The secondary efficacy outcomes were survival, tracheotomy, total MRC score, FVC, and the CGI score reported by the patient.

## **Adverse events**

The investigator observed patients for adverse events every 3 months after treatment and instructed patients to report any events at any time. After patients routinely or prematurely terminated the study,

adverse events were monitored for 3 months.

## Statistical analysis

The qualitative factors among the baseline data were analyzed using the  $\chi^2$  test, and the quantitative data were analyzed using the two independent-samples t test. The outcome measures were the changes in the ALSFRS-R score, percentage of predicted FVC, total MRC score, and CGI score from baseline to 12 months. To estimate the treatment effects, comparisons between groups were performed using the Mann–Whitney U-test for two sets of independent samples, as the data did not follow a normal distribution. Statistical analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). All statistical tests were two-sided, and a value of  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

### Patient recruitment

Between November 23, 2015 and November 22, 2017, 312 ALS patients were enrolled and randomly allocated to either the NBP group or placebo group. The last patient was followed up on November 24, 2018. The study flow chart is shown in Figure 1. (A: placebo group, B: NBP group). After the 12-month visit, the data from 93 patients in the NBP group and 92 patients in the placebo group were analyzed.

### Baseline data

Baseline data were collected at the first visit before treatment. The demographic and clinical features are shown in Table 1. Both groups had a similar sex ratio, age at first visit, duration from disease onset, site of onset, bulbar involvement at first visit, body mass index, ALSFRS-R score, and predicted FVC.

### Primary outcome

Changes in the ALSFRS-R score between baseline and the last visit of both groups are listed in Table 2. There was no difference in the ALSFRS-R score between the two groups.

### Secondary outcomes

Survival and tracheotomy: After 12 months of treatment, 13 of 93 patients in the NBP group had died and 2 had undergone tracheotomy; 6 of 92 patients in the placebo group had died and none had undergone tracheotomy ( $\chi^2 = 4.24$ ,  $P = 0.0394$ ).

After 12 months of treatment, the changes in endpoint parameters between baseline and the 12-month visit were determined, no difference was detected between the two groups in total MRC score and GCI (see Table 2). There was a mild trend of less decrease in percentage of predicted FVC in the NBP group than in the placebo group although there was no significant statistical difference.

## Adverse events

Adverse events were reported in 56.5% of patients in the placebo group and 68.8% of patients in the NBP group ( $\chi^2=2.99$ ,  $P=0.0838$ ). No severe adverse events related to the treatment were reported. Adverse events with an incidence rate greater than 1% included increases in creatinine kinase, alanine aminotransferase, and aspartate transaminase levels, gastrointestinal symptoms, lower limb edema, dizziness, respiratory symptoms, and rash (Table 3).

## Discussion

In this randomized, placebo-controlled, double-blind trial of NBP therapy in ALS patients, no intergroup difference was observed in the change in ALSFRS-R score after 12 months of treatment. There was also no difference between the two groups for some secondary outcomes, including the total MRC score and CGI scale.

A comparison of the changes in the percentage of predicted FVC during 12 months of treatment showed that this parameter decreased more slowly in the NBP group than in the control group. This result suggested that NBP might have a potential effect on slowing the decrease of respiratory function. However, this result was inconsistent with the higher mortality and tracheotomy rate in the NBP group after 12 months of treatment. The potential mechanism responsible for slowing the decrease in FVC during the 12 months of treatment needs to be further explored. The results should be interpreted cautiously because more than half of the patients in the two groups could not complete the FVC test at the 12-month visit because of bulbar muscle weakness. Those patients might have also had more serious respiratory muscle weakness in addition to bulbar muscle weakness. Therefore, the % FVC might not be a good outcome parameter to evaluate respiratory muscle function in ALS patients. Although we only recruited patients with limb symptoms at onset, most patients developed bulbar muscle weakness during follow-up. Nasal sniff pressure has been suggested as a better assessment of respiratory function in ALS patients because it can be completed in patients with bulbar muscle weakness (6).

In ALS patients, earlier treatment results in a greater benefit. In this study, we recruited ALS patients with onset duration of less than 18 months. To avoid dropout of patients because of difficulty in swallowing medicines and prevent the patients from aspiration, we only recruited patients with limb onset, and these patients exhibit longer survival. However, in this condition, it is difficult to determine the survival and tracheotomy incidence, which comprise the gold standard outcome for ALS patients. In this study, 88.6% of patients were surviving at the last visit. Although the mortality and tracheotomy rate was slightly higher in the NBP group, longer follow-up of survival and tracheotomy incidence should be performed to avoid sample bias.

In this clinical trial, we defined the ALSFRS-R score as the primary endpoint, which has been adopted in other clinical trials (7–12). However, the ALSFRS-R score might not be sufficiently sensitive to reflect mild deterioration in ALS patients. In most studies, the ALSFRS-R score slowly decreased during follow-up

among all patients, but for individual patients, 25% of 3,132 patients did not exhibit decline over 6 months. Over 12 months, 16% of 2,105 patients did not exhibit decline (13). A more efficient and accurate scale for evaluating the changes in ALS patients should be recommended for future clinical trials, especially for patients with slow progression. However, this study only compared the conditions after 12 months of treatment, which might affect the evaluation of the benefit of NBP in ALS patients, and a longer follow-up time might provide more information.

Because Riluzole and Edaravone are expensive and are not covered by the medical insurance in most provinces in China, many patients could not afford them. Furthermore, we had no information about whether NBP affect the pharmacokinetics and action of Riluzole or Edaravone, patients always doubt whether NBP decrease the effect of those medicine. So, no Patients received Riluzole or Edaravone treatment in this study.

Although NBP treatment of SOD1 mice showed benefits including delayed disease onset and longer survival (2, 3), we found no definite benefit of NBP treatment in ALS patients. This phenomenon was common in past clinical trials. The loss of motor neurons in ALS patients is insidious; almost one-third of motor neurons degenerate before weakness is presented. Therefore, initiation of treatment after several months of disease progression might be too late.

## **Conclusion**

In summary, we found no effect of NBP treatment on ALS patients according to the primary and most secondary outcome parameters, except for a mild trend of less decrease in percentage of predicted FVC.

## **Abbreviations**

ALS: amyotrophic lateral sclerosis

ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale

CGI: clinical global impression

FVC: Forced vital capacity

MRC: Medical Research Council

NBP: DL-3-n-butylphthalide

## **Declarations**

**Ethics approval and consent to participate**

All patients provided written informed consent. The ethics committee of Peking Union Medical College Hospital approved the study protocol. No animal was used in this study.

### **Consent for publication**

All the authors have approved the manuscript.

### **Availability of data and materials**

The datasets used and/or analysed in the current study are available from the corresponding authors on reasonable request except those with privacy or ethical restrictions.

### **Competing interests**

The DL-NBP in ALS clinical trials were funded by CSPC NBP Pharmaceutical Co. Ltd.

### **Funding**

This work was supported by CSPC NBP Pharmaceutical Co. Ltd.

### **Authors' Contributions**

Liyang Cui conceived and designed the experiments and reviewed and revised the manuscript; the clinical trial was performed at 19 ALS Clinical Centers; Jia He designed the protocol and input, checked, and analyzed the data; Mingsheng Liu wrote the manuscript.

### **Acknowledgements**

We thank all participating patients and their family members and the participating staff at all study sites. We thank Lisa Kreiner, PhD, from Liwen Bianji, (Edanz) ([www.liwenbianji.cn/](http://www.liwenbianji.cn/)), for editing the English text of a draft of this manuscript.

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## Tables

Table 1. Baseline characteristics of patients with amyotrophic lateral sclerosis in the two groups

|  | NBP (n=93)  | Placebo(n=92) |                | p value |
|--|-------------|---------------|----------------|---------|
| gender ratio(M/F)                        | 61/32       | 70/22         | $\chi^2=2.46$  | 0.1164  |
| Age at The first visit                   | 54.87±10.39 | 52.80±10.20   | Z=1.35         | 0.1778  |
| ration from onset (Day)                  | 11.9±4.6    | 11.6±4.3      | Z=0.44         | 0.6618  |
| Site of onset (upper/lower/both limb)    | 38/10/45    | 37/10/45      | $\chi^2 =0.01$ | 0.9960  |
| Bulbar involved at the first visit (Y/N) | 37/56       | 34/58         | $\chi^2 =0.16$ | 0.6924  |
| BMI                                      | 23.84±3.37  | 23.70±3.41    | Z=0.14         | 0.8855  |
| ALS-FRS                                  | 40.58±3.88  | 40.49±4.28    | Z=0.06         | 0.9549  |
| FVC                                      | 95.36±13.88 | 91.82±15.01   | Z=1.65         | 0.0985  |

Table 2. Analysis of the difference in ALSFRS-R, Total MRC, %FVC, GCI from baseline to the last visit between two groups.

|                           | Between-Group Difference |             |              |             | p value |
|---------------------------|--------------------------|-------------|--------------|-------------|---------|
|                           | DL-NBP                   | Placebo     |              |             |         |
|                           | Mean±SD                  | Mean±SD     | LS Mean±SE   | 95%CI       |         |
| ALSFRS-R (76/84 )         | 11.24±8.03               | 11.93±6.97  | -0.69±4.01   | -3.03,1.65  | 0.5606  |
| Total MRC score ( 55/63 ) | 25.05±16.30              | 25.16±15.03 | -0.10±158.25 | -5.82,5.61  | 0.9713  |
| %FVC ( 43/39 )            | 11.40±17.83              | 18.73±18.09 | -7.34±4.28   | -15.24,0.57 | 0.0335  |
| CGI ( 67/ 71 )            | -1.54±1.09               | -1.27±1.15  | -0.27±0.27   | -0.65,0.11  | 0.2740  |

ALSFRS-R: Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; CGI: clinical global impression scale reported by the patient; LS: least-squares; %FVC: percentage of predicted forced vital capacity; Total MRC score: total Medical Research Council score

Table 3. Most common adverse events in the two groups

| Adverse event      | NBP group (n=93) | Placebo group (n=92) | p value |
|--------------------|------------------|----------------------|---------|
| diarrhea           | 2(2.15%)         | 2(2.17%)             | 1.0000  |
| dry mouth          | 2(2.15%)         | 1(1.09%)             | 1.0000  |
| lower limb edema   | 2(2.15%)         | 1(1.09%)             | 1.0000  |
| Dizzy              | 2(2.15%)         | 3(3.26%)             | 0.9902  |
| Cough              | 1(1.08%)         | 2(2.17%)             | 0.9925  |
| oropharyngeal pain | 1(1.08%)         | 2(2.17%)             | 0.9925  |
| rash               | 1(1.08%)         | 3(3.26%)             | 0.6056  |
| ALT elevated       | 18(19.35%)       | 11(11.96%)           | 0.1664  |
| AST elevated       | 4(4.30%)         | 7(7.61%)             | 0.3415  |
| CK elevated        | 14(15.05%)       | 17(18.48%)           | 0.5329  |

## Figures

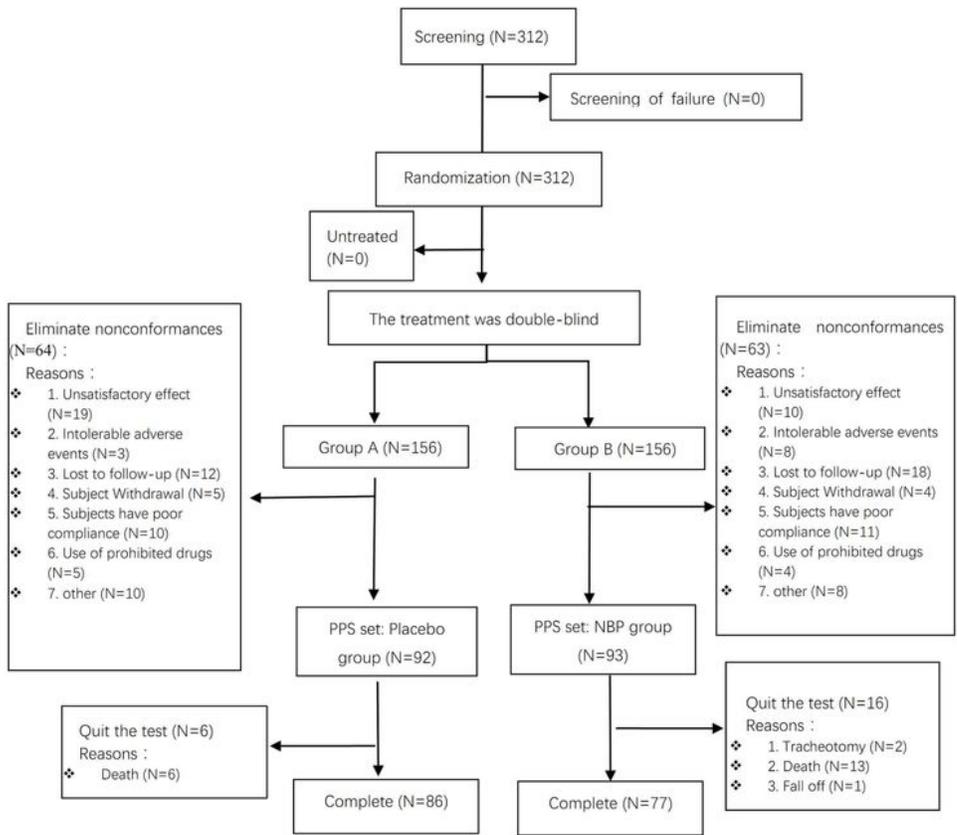


figure 1. Sample diagram of subject distribution

Figure 1

Diagram of subject distribution