Traditional Chinese medicine, BNG-1, in the recovery of ischemic stroke

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Introduction

Cerebrovascular disease is the second leading cause of death in Taiwan and accounts for the greatest number of hospitalizations for neurological diseases. Many advances occurred towards the goal of developing effective therapies to treat acute ischemic stroke. Recent studies have demonstrated that thrombolytic therapy, including intravenous thrombolytic therapy, intraarterial thrombolytic treatment, antiplatelet, antithrombotic, and neuroprotective treatments may give some beneficial effects on outcome of ischemic stroke, however, there is still controversy. The most important is recombinant tissue plasminogen activator (rt-PA) which has been approved by the Food and Drug Administration in June 1996 as a safe and effective treatment for ischemic stroke if it is given within three hours after the onset of stroke. Although rt-PA showed for the first time that acute ischemic stroke could be successfully treated, several limitations exist.

Huo Xie Shen Nao Powder (BNG-1) is a formulation of traditional Chinese medicines, consisting of 4 major (Scutellariae Radix, Angelicae Radix, Glycyrrhizae Radix, Astragali Radix) and 4 minor components, and has been used clinically to treat the stroke patient. Previous

experiments have shown that BNG-1 inhibits arachidonic acid-induced platelet aggregation and prolong bleeding time. The acute general pharmacological effects in several animal models via oral administration and in isolated guinea pig ileum assays revealed that BNG-1 exhibited no major effects on general behavior, autonomic change, and neurological, cardiovascular, respiratory, gastrointestinal and renal system. Ingestion of BNG-1 did not cause any observable acute pharmacotoxic effects in treated SD rats. On the basis of these evidences, therefore, we wish to evaluate the efficacy and safety of BNG-1 and to compare with placebo in experimental ischemic animal and in clinical trial in ischemic stroke patients.

Materials and methods

Animal study

Permanent occlusion of the left middle cerebral artery on male Sprauge Dawely rats, weighing 180-240 grams and aged 10 weeks, was used as the animal model of focal cerebral ischemia. BNG-1 was provided as a dried powder by Braingenesis Biotechnology, Ltd, and after dissolving in saline as vehicle was administrated at doses of 1000 mg/kg orally in a dosing volume of 10 ml/kg daily immediately after the surgery. The vehicle-control group was treated similarly with saline alone. After the rats were decapitated, the brains were rapidly removed, frozen in dry ice immediately, and preserved at -70°C deep freezer.

For histopathological examination of the infarction area, the rats were decapitated at the seventh day after the ischemic insult (n= 10 for BNG-1 and vehicle groups). The brains were cut as

coronal section at a thickness of 30 μm by using a microtome. Every 13th section (i.e. 390 μm apart) totally covering the infarction area of 12 mm length was selected for staining by 2% cresyl violet, and the infarcted area was accessed by an image analyzer. The infarcted volume (mm^3) was calculated by infarcted area (mm^2) × specific distance (390 μm) and then expressed as the mean ± SEM for each experimental group. The infarcted volume was compared between the BNG-1 and vehicle groups using unpaired Student's *t* test, and difference was considered significance at P< 0.05.

For immunohistochemical study of brain-derived neurotrophic factor (BDNF), another group of rats were decapitated at 4 hours, 1, 3, 7 and 28 days after ischemia (n=4 in each time point). Brain sections were cut at a thickness of 20 μm and collected on the slide glasses coated with silane (Dako, Denmark). Avidin-biotin peroxidase (ABC) method with a kit (PK-6101, Vector Lab, Burlingame, CA, USA) was used for immunostaining, and a rabbit polyclonal antibody against BDNF (1/1000, Santa Cruz Biotech, CA, USA) was used as primary antibody. Quantitation of the BDNF immunoreactive cells in both the peri-infarcted penumbra cortex and the contralateral nonischemic cortex was calculated with an image analyzer. The number difference of the BDNF immunoreactive cell between the BNG-1 and the vehicle groups were statistically analyzed using Student's *t* test. A difference of P <0.05 was considered statistically significant.

Clinical trial

This was a multi-center, phase II, double-blind, randomized, placebo-controlled,

parallel-group study. Inclusion and exclusion criteria were as follows.

Inclusion criteria:

(1) Patients of both genders (male and female).

(2) Age between 40-79 years old.

(3) No previous history of stroke or previous stroke with modified Rankin Scale ≤ 1.

(4) Patients with ischemic stroke in the cerebral hemisphere within 10 days from onset. This diagnosis was established by a physician with expertise in the diagnosis of stroke and the use of CT or MRI scan of the brain, assessed by physicians with expertise in reading these imaging devices.

(5) Patients had a clinical deficit affecting motor, perceptual, or language functions and had a total National Institute of Health Stroke Scale (NIHSS) score of 8~20 at baseline.

(6) All patients or their legal representatives provided written informed consent before participating.

(7) Female patients with negative pregnancy tests.

Exclusion Criteria:

(1) Patients with a history of other organic cerebral disease within the previous 5 years requiring hospitalization or neuroleptic therapy.

(2) Patients with significant impairment of renal function (BUN > 1.5 times of the upper limit of normal range or creatinine > 3 mg/dl); severe liver injury (SGOT and SGPT above double upper limit of normal); severe cardiac disease (New York Heart Association Functional

Classification III and IV) or currently under investigation or treatment of any carcinoma.

(3) Patients with another stroke except ischemic stroke or a serious head injury, as well as alcoholism and/or drug abuse in the previous 3 months.

(4) Female patients who were pregnant, lactating or suspected for possible pregnancy.

(5) Patients who have participated in another clinical study within the previous 1 month.

(6) Patients with insulin-dependent diabetes mellitus (IDDM) or a.c. sugar ≥ 200 mg/dl after treatment for non-insulin dependent diabetes mellitus (NIDDM).

(7) Post-treatment systolic blood pressure greater than 185 mmHg or diastolic blood pressure greater than 110 mmHg.

(8) Patients who were allergic to aspirin.

(9) Patients who had received concomitant medication with Hydergine, Nootropil, Ginex, Trental, Sermion within one month prior to the study or during the study.

(10) Platelet count $< 100 \times 10^3 / mm^3$

In this double-blind study, we compared mainly the safety of orally administered BNG-1 9 g daily to placebo in ischemic stroke patients in concomitant with aspirin 100 mg qd. Each patient was randomly assigned to receive aspirin 100 mg qd + BNG-1 3 g/ pack tid or aspirin 100 mg qd + placebo 3 g/ pack tid after meals for 14 days. They were then followed by a 24-week follow-up phase for safety assessment. The rehabilitation program was initiated as soon as possible, and when the condition was stable, the bedside exercise and other rehabilitation programs were applied immediately on both test and control groups. The safety was evaluated, including all abnormal data

found in vital signs, physical examination, and laboratory examination, and all adverse events that might have happened during the study period. Any adverse event, spontaneously reported or observed by the research team during the treatment, was recorded. Adverse events were recorded in response to an open, standardized questionnaire conducted at each visit.

Results

Animal study

In the histopathological study, the present result showed that the total infarcted volume in the BNG-1 treated group (62.14 ± 10.73 mm^3) was significantly smaller than the vehicle treated group (115.7 ± 14.4 mm^3) ($P < 0.05$) at the seventh day after permanent occlusion of left middle cerebral artery.

In the immunohistochemical study of BDNF, the present study showed that from 4 hours to 28 days after cerebral ischemia, there was no significant difference of the number of BDNF-immunoreactive cell in right nonischemic cortex between the BNG-1 and the vehicle groups ($P > 0.05$). However, in the left ischemic cortex, there was a significant reduction of BDNF-immunoreactive cell number at 4 hours and 1 day after cerebral ischemia in both the BNG-1 and vehicle groups ($P < 0.05$). At 7 days, the ischemic cortex had a significant increase of BDNF-immunoreactive cell number when compared with the nonischemic cortex in BNG-1 group ($P < 0.05$), but not in vehicle group ($P > 0.05$). Also, the BNG-1 group had a significant increase of BDNF-immunoreactive cell number when compared with that of the vehicle group in the ischemic

cortex ($P< 0.05$). At 28 days after cerebral ischemia, there was no significant difference of BDNF-immunoreactive cell number between the BNG-1 and the vehicle groups ($P> 0.05$).

Clinical trial

The percentage of patients reporting an adverse event was comparable between the two treatment groups. The total incidence of adverse events (AEs) was 179 events in the BNG-1 group and 151 events in the placebo group. For all-treated patients, the percentage of patients reporting an adverse event in the BNG-1 group was 95.5% (21/22) as compared with 90.5% (19/21) in the placebo group with no statistically significant difference ($P=0.607$). There was no significant difference between these two groups with respect to the following: number of patient who terminated early in the study due to AEs, the incidence of serious adverse events (SAEs), and the number of patients with treatment-related AEs, or SAEs. The only suspected treatment-related AE was ecchymosis, which occurred in the control group after 1 week of treatment and resolved at the end of treatment. No patient in either group was discontinued from the study due to AEs.

Discussion

The present study suggests that the traditional Chinese medicine, BNG-1, may have a protective effect on the ischemic cortical neurons against focal cerebral ischemia. As BDNF is known to be protective on the cortical and hippocampal neurons against ischemia, it is possible that the protective effect of BNG-1 may act through the neurotrophic system. The clinical trial showed that there were no significant difference between the two groups with respect to AEs, serious AEs,

discontinuation due to AEs, and the number of patients with drug-related AEs. The small sample size of the present clinical trial may have rendered the efficacy results difficult to interpret. A phase III clinical trial with a larger sample size to compare the functional outcome and safety of BNG-1 with placebo in ischemic stroke will be undertaken.