BNG-1 in the Recovery of Acute Ischemic Stroke: A phase 2, Double-blind, Placebo-controlled Randomized Trial

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Abstract-

Purpose: Few strategies have been approved for acute therapy of ischemic stroke in Western medicine. Hundreds of traditional Chinese medicines (TCMs) have been used for stroke therapy and were rarely tested by qualified studies. To evaluate the efficacy and safety of BNG-1, a novel mixture of TCMs, in patients with acute ischemic stroke, we conducted the clinical trial.

Methods: This was a Phase 2, double-blind, placebo-controlled study in which the safety and efficacy of orally administered BNG-1 based on oral aspirin 100 mg daily for consecutive 14 days were measured in patients with acute ischemic stroke within 10 days after onset. The primary efficacy endpoint was the functional status assessed by the Barthel Index. The safety was evaluated by the incidence of adverse events and significant changes in vital signs, parameters of physical and laboratory examinations.

Results: There were 42 patients randomized for the intention-to-treat efficacy analysis. The study failed to prove the significantly statistical difference of efficacy assessment between patients receiving BNG-1 and placebo in the recovery of acute ischemic stroke. The clinical and laboratory safety profiles had no significant difference between two groups.

Conclusions: BNG-1 trial was feasible, safe and well tolerated for patients with acute ischemic stroke based on the treatment of aspirin, though there was no statistically significant difference of efficacy between BNG-1 and placebo groups. A further large Phase 3 trial of BNG-1 is needed before recommending such treatments for general clinical use.

Key Words: acute ischemic stroke; traditional Chinese medicine; BNG-1; randomized control trials.

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INTRODUCTION

Stroke is the leading cause of adult neurological disability and accounts for the greatest number of hospitalizations for neurological diseases worldwide. In Taiwan, it has been the second or third leading cause

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of death and also the leading cause of serious long-term disability with an annual crude mortality rate 47.5 per 100,000 population in 2012[1]. In a decade, the only effective therapy to treat acute ischemic stroke approved by the Food and Drug Administration was the recombinant tissue plasminogen activator as a safe and effective treatment for stroke if it is given within three hours after the onset of stroke[2], although several limitations exist[3]. Subsequently, clinical trials of antiplatelet, antithrombotic, or neuroprotective treatments for acute ischemic stroke were not successfully to demonstrate the efficacy on poststroke outcome[4].

More than 100 traditional Chinese medicines (TCMs) have been approved for stroke therapy in China[5], and some products are also popularly prescribed by the physicians of TCMs in Taiwan. Although few studies had good qualified clinical design and conduction to conclude the efficacy[5], however, none of them showed impact to clinical outcome in patients with ischemic stroke. A large number of compounds have been isolated from TCMs and most of these resources have not yet been characterized for pharmacological purposes[6]. Therefore, it is significant to explore potential Chinese medicinal therapy from pharmacological perspective.

Huo Xie Shen Nao Powder (BNG-1), as its name denotes in Chinese to help eliminating blockages and stagnation, and nourishing the brain, is composed of eight medicinal herbs, i.e., Tang Kuei, Ginseng, Scutellaria, Coptis, Glycyrrhiza, Astragalus, Bamboo Sugar and Bupleurum. It was developed by an experienced Chinese medicine practitioner Dr. Deng Fa Huang, with more than 30-year clinical experience in the treatment of stroke. BNG-1, a novel mixture of TCMs with a long history in the treatment of stroke, exhibited acute neuroprotection effect on rats with middle cerebral artery occlusion[7,8]. Anti-ischemic effects were seen in both animals receiving BNG-1 before the ischemic insult as well as in animals receiving the drug formulation after surgical occlusion of the middle cerebral artery[7,8]. Antithrombotic activity was seen in vitro to inhibit arachidonic acid-induced platelet aggregation and in vivo to prolong bleeding time in mice[9]. BNG-1 was also found to inhibit several phosphodiesterase (PDE) isoforms with potency order of the following rank: PDE 1 > PDE 3 > PDE 6 > PDE 2 > PDE 4 > PDE 5.[7] Other pre-clinical results and emerging clinical data coupled with the present findings suggest that BNG-1 may be a safe and effective therapy for both the prevention and treatment of ischemic stroke. Moreover, the fundamental cellular mechanism underlying its therapeutic effects may result from PDE inhibition. To evaluate the safety and efficacy of BNG-1 in comparison with placebo based on the treatment of aspirin in acute ischemic stroke patients, we conducted the clinical trial.

METHODS

Protocol Design

This was a randomized double-blind, placebo-controlled, parallel comparative Phase 2 study in which the safety and efficacy of orally administered BNG-1 were measured in patients with onset of acute ischemic stroke within 10 days in Chang Gung Memorial Hospital, Linkou and Kaohsiung, Taiwan. This is the first scientific clinical trial of TCMs followed the guideline for Good Clinical Practice in Taiwan. The treatment and follow-up schedule was displayed in Figure 1. In addition to taking oral aspirin 100 mg daily in both groups, the treatment group took 3 g/pack of BNG-1 and the control group had 3 g/pack of placebo orally after meal, three times per day, for consecutive 14 days. A follow-up phase was 24 weeks after stop of study drug administration. The primary efficacy endpoint was the functional status assessed by the Barthel Index (BI). The secondary efficacy endpoints were neurological recovery assessed by the National Institutes of Health Stroke Scale (NIHSS), and level of disability based on the modified Rankin Scale (MRS). The safety was evaluated by the incidence of adverse events (AEs) and significant changes in vital signs, parameters of physical and laboratory examinations, including hematology (red blood cell count, white blood cell count, platelet count, differential count, hemoglobin, hematocrit, erythrocyte sedimentation rate, bleeding time, fibrinogen, prothrombin time test and partial thromboplastin time test) and biochemistry (total protein, albumin, total bilirubin, AST, ALT, BUN, creatinine, sodium, potassium and chloride), were performed at visits 1 to 9.

The protocol was reviewed and approved by the Institution Review Board of Chang Gung Memorial Hospital and Department of Health, Executive Yuan, Taiwan. The signed informed consent was obtained from

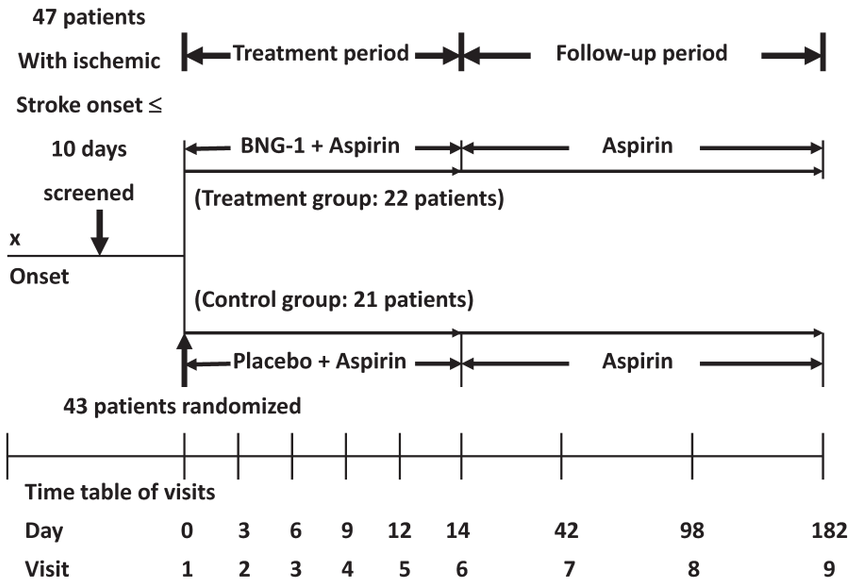

Figure 1. Schematic diagram of treatment and follow-up schedule. Vital signs, laboratory tests, concomitant medications and adverse events were evaluated at each visit. Physical examinations, National Institute of Health Stroke Scale, Barthel Index and modified Rankin Scale were documented for analysis at visits 1, 3 and 6-9.

Table 1. Inclusion and exclusion criteria of the enrolled patients

Inclusion Criteria:

1. Patients of both genders (male and female).
2. Age between 40 and 79 years old.
3. No previous history of stroke or previous stroke with modified Rankin Scale ≤1.
4. Patients with the ischemic stroke in cerebral hemisphere within 10 days from onset. This diagnosis was established by a physician with expertise in diagnosis of stroke and computed tomography or magnetic resonance imaging scan of the brain was assessed by physicians with expertise in reading this imaging study.
5. Patients had a clinical deficit affecting motor, perceptual, or language functions and had a total National Institutes of Health Stroke Scale 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 five years requiring hospitalization or neuroleptic therapy.
2. Patients with significant impairment of renal function (blood urea nitrogen > 1.5 times of the upper limit of normal range or creatinine > 3 mg/dl); severe liver injury (aspartate aminotransferase and alanine aminotransferase 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 three months.
4. Female patients who were pregnant, lactating or suspected for possible pregnancy.
5. Patients who had participated in another clinical study within the previous one month.

Table 1. Inclusion and exclusion criteria of the enrolled patients (Continued)

6. Patients with insulin-dependent diabetes mellitus or fasting sugar ≥ 200 mg/dl after treatment for non-insulin dependent diabetes mellitus.
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 (Dihydroergotoxine mesylate), Nootropil (Piracetam), Ginex (Ginkgo Biloba Extract), Trental (Pentoxifylline), Sermion (Nicergoline) within the previous one month or during the study.
10. Platelet count $<100\times10^3$ / mm^3

each patient before enrollment. The study period was from August 27, 2001 to February 06, 2003. Patients fulfilled the inclusion criteria and excluded by the exclusion criteria were enrolled (Table 1).

Methods of Analysis

Data analyses of the efficacy and safety endpoints were performed for the intention-to-treat (ITT) population-all randomized patients who took study medication and had at least another evaluation regardless of their compliance with the protocol. The primary hypothesis was that the distribution of subjects of study drug (BNG-1) in response categories is superior to reference drug (placebo). All hypothesis tests were conducted at the 5% level of significance. Continuous variables were analyzed by t-test or analysis of variance (ANOVA). Fisher's exact test, chi-square test, and Wilcoxon rank-sum test were used to compare between the two treated groups for categorical variables, including safety profiles. For the primary efficacy endpoint (BI) and secondary efficacy endpoints (NIHSS and MRS), comparison of two treatment groups was made at the end of the study and changes from baseline to the endpoint visit. Following statistical methods were used to evaluate the efficacy endpoints: BI and NIHSS were evaluated with Wilcoxon rank-sum test; MRS, Fisher's exact test and CMH-test (for change-from-baseline).

RESULTS

The recruited procedure was shown in Figure 2 and there were 42 patients included for the ITT analysis. No significant differences were found in demographics and baseline characteristics between the two treatment groups (Table 2).

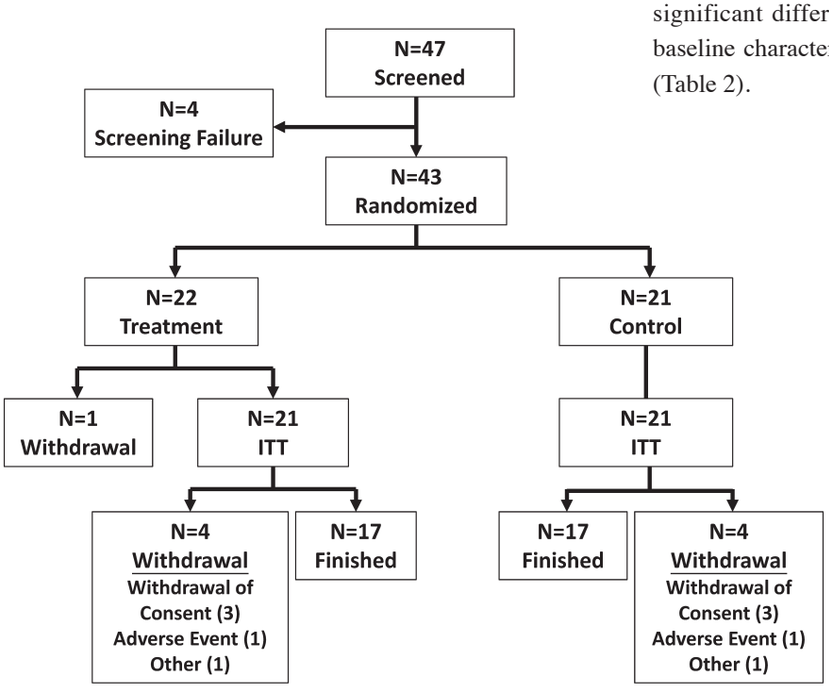

Figure 2. Disposition of patients. There were 42 patients randomized in the intention-to-treat (ITT) analysis with 21 patients in each group, after excluding one patient randomized to the treatment group with only one dose of medication and without any follow-up assessment due to condition change favored disease natural course.

Table 2. Summary of demographic and baseline characteristics for intention-to-treat population

Characteristics	Treatment (N=21)	Control (N=21)	P-value (95% C.I.)
Age (years)			
Mean (SD)	63.8 (9.6)	64.2 (9.4)	0.906*
Median (Range)	65.3 (40.3, 77.7)	65.9 (45.4, 77.8)	
Sex (n %)			0.756†
Female	10 (47.6%)	8 (38.1%)	
Male	11 (52.4%)	13 (61.9%)	
Barthel Index			
Mean (SD)	30.5 (23.1)	22.4 (19.2)	0.086‡
Median	30.0	20.0	
NIHSS Score#			
Mean (SD)	11.9 (3.5)	12.8 (4.2)#	0.393‡
Median	11.0	11.0	
Preexisting condition (n %)			
Aspirin therapy	21 (100 %)	21 (100 %)	
Atrial fibrillation	3 (14.3 %)	0 (0.0 %)	
Hypertension	13 (61.9 %)	13 (61.9 %)	
History of stroke	3 (14.3 %)	1 (4.8 %)	
Diabetes mellitus	9 (42.9 %)	12 (57.1 %)	
Valvular heart disease	1 (4.8 %)	0 (0.0 %)	
Cardiomegaly	0 (0.0 %)	1 (4.8 %)	
Hyperlipidemia§	2 (9.5 %)	6 (28.6 %)	
Thrombocytopenia	1 (4.8 %)	1 (4.8 %)	

* Two sample t-test.

† Fisher's exact test.

‡ Wilcoxon rank sum test.

N=20 One patient was blind in nature, and was excluded from National Institutes of Health Neurological Stroke Scale (NIHSS) analysis.

§ Hypercholesterolemia and hypertriglyceridemia included.

Efficacy Results

The trends of BI and NIHSS scores revealed significant improvement ($p < 0.05$) as compared with those of the baseline in both groups, though there was no statistically significant difference ($p > 0.05$) between BNG-1 and placebo groups at each visit's evaluations (Figs. 3A and 3B, and Table 2). The differences of all visits of MRS evaluations between the two groups were also insignificant (Fig. 4, $p > 0.05$). At baseline, most of the patients were severely disabled (MRS = 4 or 5), 90% (19/21) in the treatment group vs. 95% (20/21) in the control group. After two weeks of treatment, more patients in the treatment group (28.6%, 6/21) retained a mild disability (MRS = 2 or 3) compared to those in the control group (9.5%, 2/21).

SAFETY RESULTS

Table 3, including one randomized patient in BNG-1 group but without any one efficacy evaluation, shows no significant differences ($p > 0.05$) in the incidence of AEs between two groups in any organ systems. Most of the AEs reported (98.3% in the treatment group and 99.3% in the control group) were of mild to moderate in severity. The most frequently reported AEs were those related to the gastrointestinal system and the nervous system or psychiatric disorders. For all-treated patients, the percentage of patients reporting an adverse event in the treatment group was 95.5% (21/22) as compared with 90.5% (19/21) in the control group with no statistically significant difference ($p = 0.607$). No patient, except for

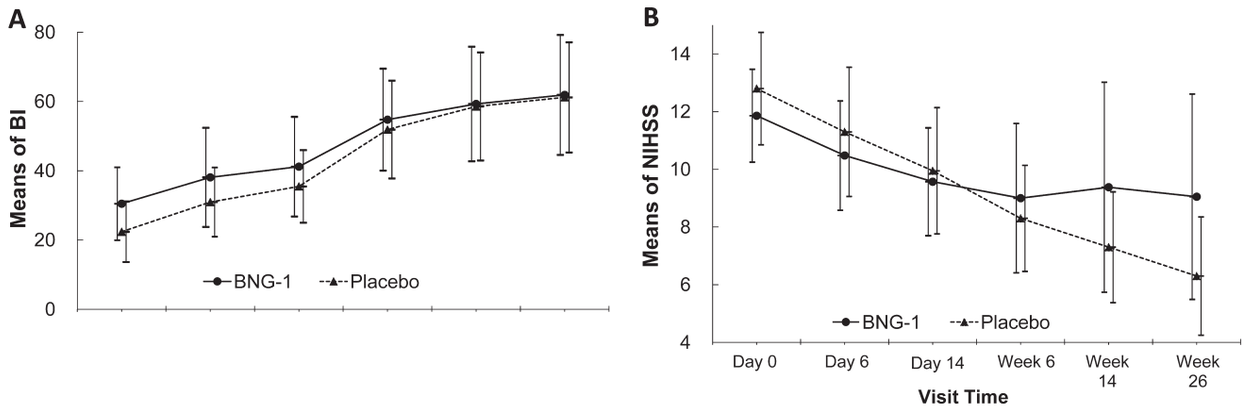

Figure 3. Trends of primary (A: Barthel Index, BI) and secondary (B: National Institute of Health Stroke Scale, NIHSS) endpoints with 95% confidence interval between BNG-1 and placebo groups during the treatment (Days 1-14) and follow-up (Weeks 2-26) periods. The scores of BI (A) and NIHSS (B) at each evaluation period were not statistically significant ($p > 0.05$) between the two groups.

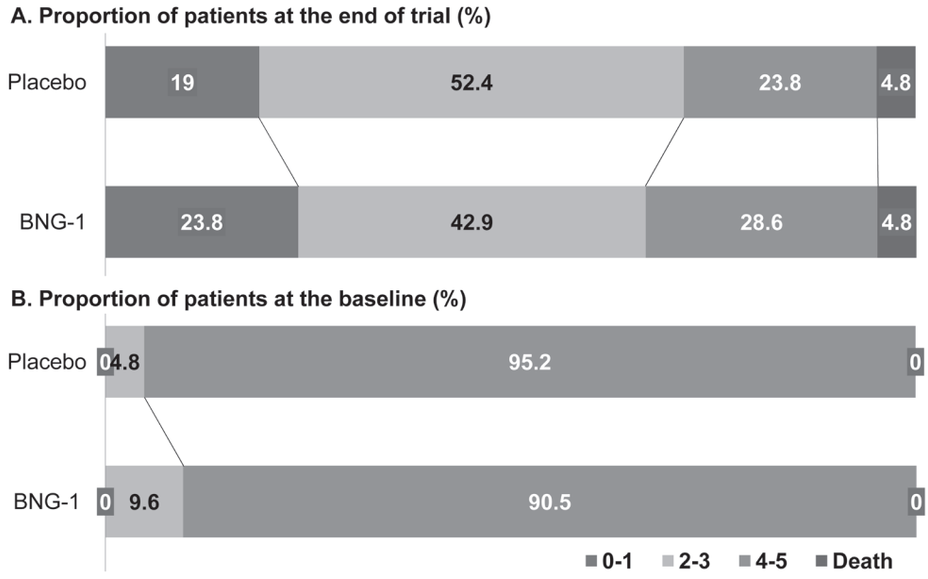

Figure 4. Shift analysis of the percentage of modified Rankin Scale. Common estimate of the odds ratio of improvement over the four grades at baseline are 0.475 (95% confidence interval, 0.040-5.679, $p = 1.000$) and end of the study (Odds ratio can't be estimated because four cells (50%) of expected numbers are less than five.) between BNG-1 and placebo groups.

Table 3. Summary of adverse events by body system

Body System	Treatment N=22†	Control N=21	P-Value*
Body as a Whole	6 (27.27 %)	10 (47.62 %)	0.215
Cardiovascular System	6 (27.27 %)	0 (0.000 %)	0.021‡
Endocrine System	3 (13.64 %)	1 (4.762 %)	0.607
Ear, Nose and Throat	8 (36.36 %)	6 (28.57 %)	0.747
Eye	2 (9.091 %)	0 (0.000 %)	0.488
Gastrointestinal System	13 (59.09 %)	12 (57.14 %)	1.000
Hematology	3 (13.64 %)	2 (9.524 %)	1.000
Metabolic and Nutritional Disorder	3 (13.64 %)	5 (23.81 %)	0.457
Muscular Skeleton System	9 (40.91 %)	8 (38.10 %)	1.000
Nervous System and Psychiatric Disorder	16 (72.73 %)	14 (66.67 %)	0.747
Respiratory System	8 (36.36 %)	5 (23.81 %)	0.510
Reproductive System	1 (4.545 %)	1 (4.762 %)	1.000
Skin	8 (36.36 %)	5 (23.81 %)	0.510
Urinary System	6 (27.27 %)	7 (33.33 %)	0.747

* Fisher's Exact Test. † Including one randomized patient in BNG-1 group but without any one efficacy evaluation. ‡p<0.05.

two mortality cases with one in each group during the follow-up period, in either group was discontinued from study due to AEs. There were no significant differences ($p > 0.05$) between the two groups with respect to AEs, serious AEs, discontinuation due to AEs, and the number of patients with drug-related AEs.

Although significant differences ($p < 0.05$) of red blood cell counts, segmented neutrophils, and alanine transaminase between treatment groups were transiently detected at a few visits, there was no clinical significance and no patient was discontinued from the study due to laboratory changes. The vital signs throughout the study were no significant differences between the two groups.

DISCUSSION

This Phase 2, double-blind, placebo-controlled randomized study of a modified TCM herbal compound BNG-1 plus aspirin versus placebo plus aspirin in patients with acute ischemic stroke has shown to be feasible and safe, although it failed to prove the significantly statistical differences of efficacy assessments, included functional status (BI), neurological recovery (NIHSS), and level of disability (MRS), between two groups. However, with regard to the level of disability, there was a trend on MRS, although not significant, showing that there were more patients who recovered from severe disability to a mild disability in the treatment group than in the control group throughout the treatment period. This trend might have partially resulted from the unbalanced distribution of patients between both groups at baseline. It was noted that more patients rated MRS 5 in the control group than those in the treatment group [10 (48%, 10/21) vs. 5 (24%, 5/21)]. This slightly unbalanced distribution (not statistically significant) of patients in treatment groups is most likely due to random effect in study involving small number of patients.

Strokes of varying severity have nonlinear profile of recovery, and the early course of improvement may be greater at the lower end of the deficit scale than at the higher end in NIHSS[10]. Patients in this study with various stroke severities at baseline are enrolled into a non-stratified group to receive either the treatment medication or the placebo. The improvements in both groups were likely to be the 'natural course of the disease', which could have diluted the potential to observe a true effect of BNG-1. Patients in the treatment group seemed to have a worse response to the therapy than those in the control group with regards to NIHSS during the treatment period.

This study involved BNG-1 co-administered with aspirin in the recovery of ischemic stroke, and therefore, it is compulsory to discuss the role of aspirin in this

study. Antiplatelet therapy is recommended for stroke prevention, and aspirin was the first antiplatelet agent to be used in this context and is still the most frequently prescribed preventive treatment for ischemic stroke[11]. Furthermore, clinical study indicated that the reduction in further stroke or death from just a few weeks of early aspirin use is 9 per 1000 patients within 1 month[12]. The effect on the recovery of functional status has not been studied yet. Thus, the improvement in functional status in this study can not be ascribed to the effect of aspirin.

Abnormal changes in RBC were observed in the treatment group, but were not supported by previous animal study results, which showed no abnormal change in hematological parameters in rats after repeated oral dose of up to 3000 mg/kg/day for 28 days[13], nor in beagles after repeated oral dose of up to 2000 mg/kg/day for 28 days[14]. These changes were not correlated with clinical conditions and none of them were considered as related to the study drug, and no patient was discontinued due to the laboratory changes. Nonetheless, the transient changes in those parameters observed in the treatment period suggested a more cautious safety monitoring plan for future studies.

The Phase 2 study had some limitations to demonstrate the efficacy of BNG-1 for acute therapy of ischemic stroke patients due to the small enrolled population, and wide distributed etiologies and presentations of ischemic stroke. In the future, efforts will be required to identify potentially responsive cases based on TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification, which includes the five major ischemic stroke groups[15]. The oral administration of the medications and later prescription of BNG-1 till 10 days after stroke onset might also limit to save the potential reversible neurons after the onset of drug effect. Because of bypassing the Phase I study, the effective timing of inclusion, dosage and duration of BNG-1 treatment should also be determined. However, based on the encouraging results from previous animal models and basic scientific studies, as well as present Phase 2 trial result of feasibility and safety, a Phase 3, double-blind randomized, placebo-controlled trial of BNG-1 with larger scale of enrolled patients is needed to investigate stroke recovery before such treatments can be recommended for general clinical use.

CONCLUSIONS

BNG-1 for patients with acute ischemic stroke based on the treatment of aspirin was safe and well tolerated, though there was no statistically significant difference of efficacy between BNG-1 and placebo in this Phase 2 trial. It needs a further large Phase 3 trial before recommending such treatments for general clinical use.

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