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

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Introduction (1)

- Cerebrovascular disease: the second leading cause of death in Taiwan and the greatest number of hospitalizations for neurological diseases.
- Thrombolytic therapy: intravenous thrombolytic therapy, intraarterial thrombolytic treatment, antiplatelet, antithrombotic, and neuroprotective treatments.
- Tissue plasminogen activator: June 1996, within three hours after the stroke onset.

Introduction (2)

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

Introduction (3)

- Inhibits arachidonic acid-induced platelet aggregation and prolong bleeding time.
- Acute general pharmacological effects: 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.

Purpose

To evaluate the efficacy and safety of BNG-1 and compare with placebo in experimental ischemic animal and in clinical trial in ischemic stroke patients.

Material and method: Animal study (1)

- Male SD rats: 180-240 grams, aged 10 weeks.
- Permanent occlusion of the left middle cerebral artery.
- BNG-1 was provided as a dried powder by Braingenesis Biotechnology, Ltd.
- BNG-1 (dissolved in saline): daily doses of 1000 mg/kg orally.
- Vehicle-control group: saline alone.

Animal study (2)
Histopathological study

- Decapitated at the seventh day after ischemia.
- Coronal brain section (30 μm), every 13th section, 12 mm length, 30 slices.
- 2% cresyl violet, image analyzer.
- Infarcted area (mean ± SEM mm^2) of each coronal slice from each animal.
- Infarcted volume (mean ± SEM mm^3) = infarcted area (mm^2) × specific distance (390 μm).
- Infarcted volume: BNG-1 vs vehicle groups

Animal study (3)

Immunohistochemical study of BDNF

- Reperfusion time points: 4 hours, 1 day, 3 days, 7 days and 28 days after ischemia.
- Brain sections: 20 μm.
- Avidin-biotin peroxidase (ABC) method.
- Quantitation of the BDNF immunoreactive cells: peri-infarcted penumbra cortex and contralateral nonischemic cortex with an image analyzer.
- BDNF immunoreactive cells: BNG-1 vs vehicle groups

Clinical trial (1)

- Braingenesis Biotechnology Co., LTD.
- Multi-center, phase II, double-blind, randomized, placebo-controlled, parallel-group study.
- Study site: Lin-Kou Chang-Gung Memorial Hospital and Kaohsiung Chang-Gung Memorial Hospital.
- Study Period: from August 27, 2001 to February 06, 2003.
- Subjects: 60 patients (40 completed the study) planned. 47 patients were screened, 43 patients were randomized, 42 were ITT Population.

Clinical trial (2):

- Medication: 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.
- Follow-up period: 24 weeks.
- Safety Endpoints: the incidence of adverse events and significant changes in vital signs, physical examination parameters and laboratory examination parameters.

Clinical trial (3): 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 $\leqq 1$.
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 CT or MRI scan of the brain was assessed by physicians with expertise in reading this imaging study.

Clinical trial (4): Inclusion criteria

5. Patients had a clinical deficit affecting motor, perceptual, or language functions and had a total National Institutes 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.

Clinical trial (5): 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.

Clinical trial (6): Exclusion criteria

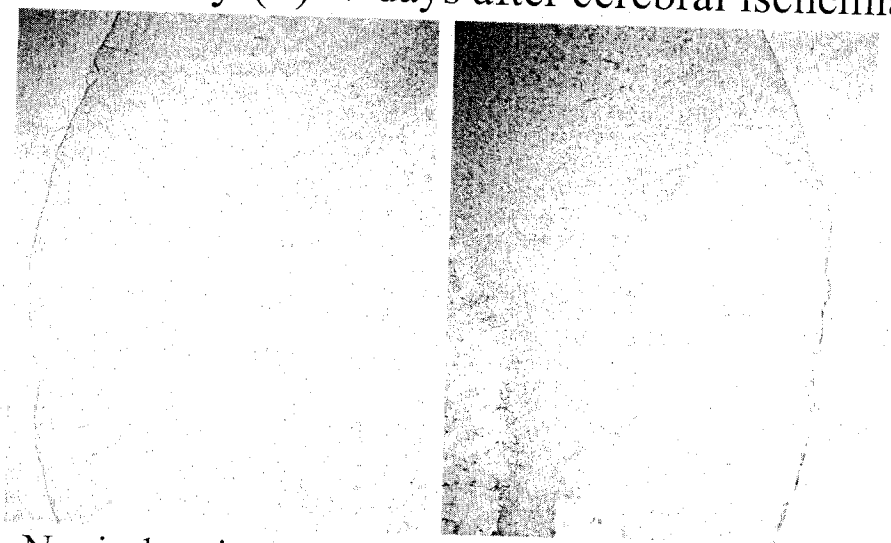
3. Patients with another stroke except ischemic stroke or a serious head injury, as well as alcoholism and/or drug abuse in the previous 3 months.
4. Female patients who were pregnant, lactating or suspected for possible pregnancy.
5. Patients who had participated in another clinical study within the previous 1 month.
6. Patients with Insulin-dependent diabetes mellitus (IDDM) or a.c. sugar $\geq$ 200 mg/dl after treatment for Non-insulin dependent diabetes mellitus (NIDDM).

Clinical trial (7): Exclusion criteria

7. Post-treatment systolic blood pressure greater than 185 mmHg or diastolic blood pressure greater than 110 mmHg.
8. Patients were allergic to aspirin.
9. Patients had received concomitant medication with Hydergine, Nootropil, Ginex, Trental, Sermion within the previous one month or during the study.
10. Platelet count $< 100 \times 10^3 / mm^3$

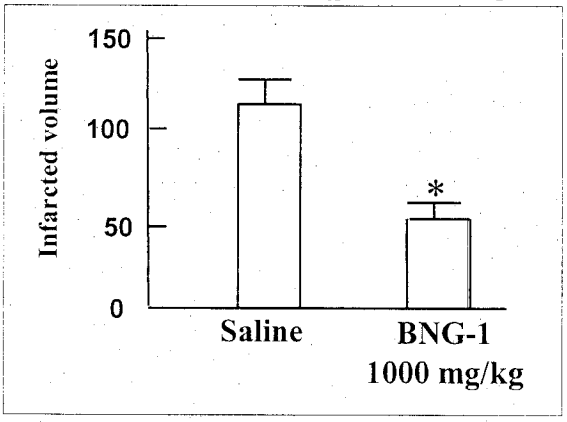
Result:

Animal study (1): 7 days after cerebral ischemia



Nonischemic cortex Ischemic cortex

Animal study (2): Histopathological study



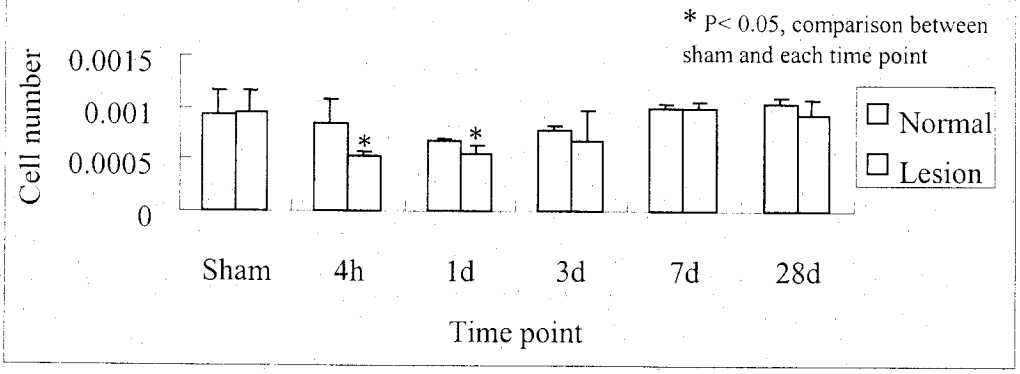
infarcted volume at 7 days after permanent occlusion of left middle cerebral artery

BNG-1 treated group (62.14 ± 10.73 mm^3)

vehicle treated group (115.7 ± 14.4 mm^3) ($P < 0.05$)

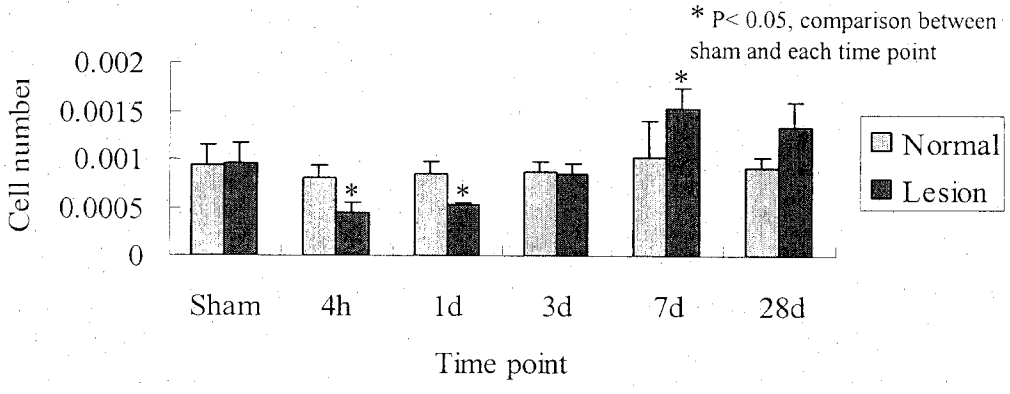
Animal study (3): Immunohistochemistry

Temporal profile of BDNF-immunoreactive cell in the normal and peri-infarcted (lesion) cortical areas after saline treatment in permanent focal cerebral ischemia of rats



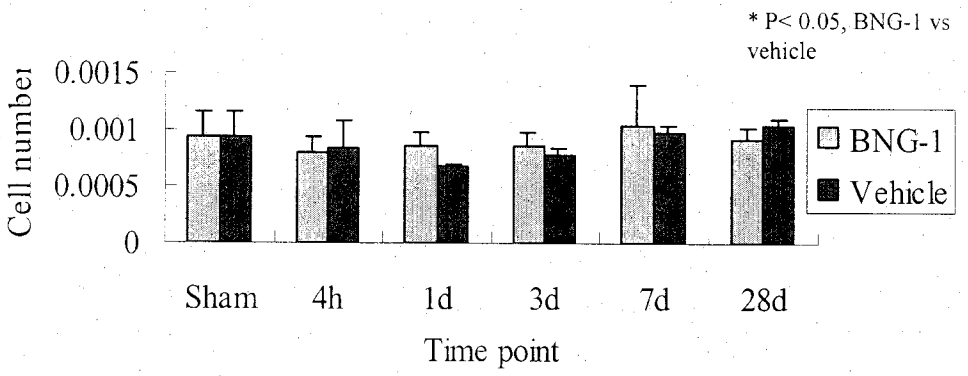
Animal study (4): Immunohistochemistry

Temporal profile of BDNF-immunoreactive cell in the normal and peri-infarcted (lesion) cortical areas after BNG-1 treatment in permanent focal cerebral ischemia of rats



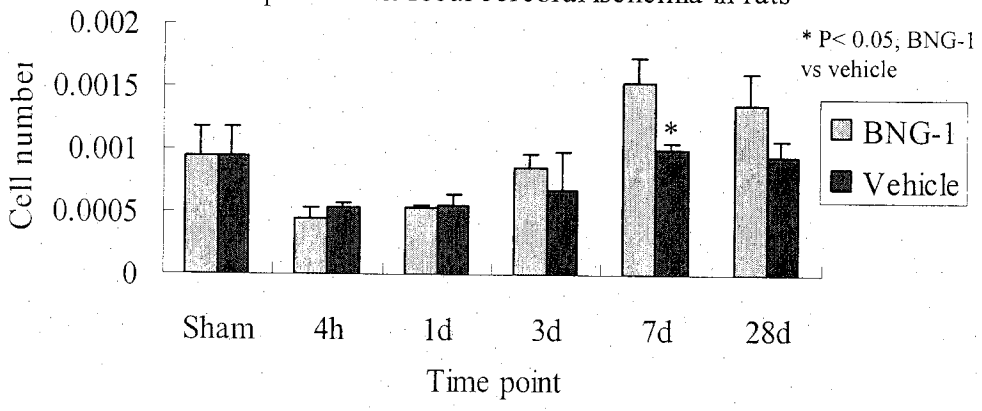
Animal study (5): Immunohistochemistry

Comparison of BDNF-immunoreactive cell number in the normal cortical area between BNG-1 and vehicle treatment after permanent focal cerebral ischemia in rats

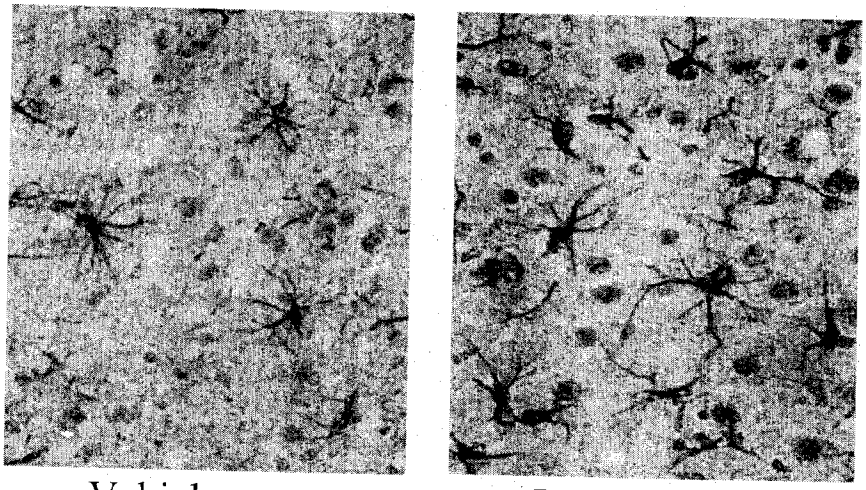


Animal study (6): Immunohistochemistry

Comparison of BDNF-immunoreactive cell number in the peri-infarcted cortical area between BNG-1 and vehicle treatment after permanent focal cerebral ischemia in rats



Animal study (7): Double immunostaining in the peri-infarcted cortical area at 7 days after cerebral ischemia



Vehicle group BNG-1 group

Blue: GFAP for reactive astrocyte; Brown: BDNF

Results: Clinical trial

Patient Disposition

Population	Number of Patients (%)	
	BNG-1	Placebo
All Screened	47	
Screen failure	4	
Randomized	22 (100.0%)	21 (100.0%)
ITT population	21 (95.5%)	21 (100.0%)
Completed the 2-week treatment	21 (95.5%)	20 (95.2%)
Completed the 3-month follow-up	20 (90.9%)	17 (81.0%)
Finished the study	17 (77.3%)	17 (81.0%)
Discontinued from the study	5 (22.7%)	4 (19.0%)
Withdrew consent	3	2
Lost to follow-up	0	1
Adverse events	1	1
Others*	1	0

*Patient's condition changed

Results: Clinical trial

Baseline Demographics and Disease Characteristics

Characteristics		BNG-1 (N=21)	Placebo (N=21)	P-value
Age (years)		63.8	64.2	0.906*
Sex (n %)	FEMALE	10 (47.6%)	8 (38.1%)	0.756#
	MALE	11 (52.4%)	13 (61.9%)	
Barthel Index (MEAN)		30.5	22.4	0.086‡
NIHSS Score (MEAN)		11.9 (3.5)	12.8 (4.2)§	0.393‡
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		9 (42.9 %)	12 (57.1 %)	
Rheumatic 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; † Hypercholesterolemia and hypertriglyceridemia included
‡ Wilcoxon Rank Sum test; § N=20 Patient No. 301was blind in nature, and was excluded from NIHSS analysis.

Safety Results

Adverse Events

Summary of adverse event (AE)

	BNG-1	Placebo	P-Value*
	N = 22	N = 21	
• Total number of AE	179	151	--
• Total patient number of AE	21 (95.5%)	19 (90.5%)	0.607
• Total number of patient who stops study drug due to AE	0	0	1.0
• Total number of patient who has AE related to study drug	0	1	0.488
• Total number of patient who discontinues the study due to AE	1	1	1.0
• Severe AE	6	6	--
• Total number of patient who has severe AE	4 (18.2%)	6 (28.6%)	0.488

*Fisher's Exact test

Body System	BNG-1 N=22	Placebo 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
Gastro Intestinal 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

Adverse Events

- Difference between treatments (by body system):
  - Cardiovascular System

Cardiovascular System	BNG-1	Placebo
COSTART Term	N=22	N=21
Number of Patient	6 (27.27 %)	0 (0.00 %)
Incidence	9	0
Hypertension*	5 (22.73 %)	0 (0.00 %)
Coronary artery disorder#	2 (9.09 %)	0 (0.00 %)
Hypotension†	1 (4.55 %)	0 (0.00 %)
Angina pectoris‡	1 (4.55 %)	0 (0.00 %)

*PN=104,115,124
#PN=130,208
†PN=134
‡PN=208

Difference between groups: P=0.014

Adverse Events

- Difference between treatments (by COSTART term)
  - Pain
    - BNG-1 : Placebo = 1/22 : 6/21 (P=0.046)

RBC	BNG-1 N (%)	Placebo N (%)	(Fisher's) P-value
Visit 1 (Day 0)			0.034
ABNORMAL	9 (40.91%)	2 (9.52%)	
NORMAL	13 (59.09%)	19 (90.48%)	
Visit 2 (Day 3)			0.484
ABNORMAL	6 (30.00%)	4 (19.05%)	
NORMAL	14 (70.00%)	17 (80.95%)	
Visit 3 (Day 6)			0.015
ABNORMAL	10 (47.62%)	2 (9.52%)	
NORMAL	11 (52.38%)	19 (90.48%)	
Visit 4 (Day 9)			0.009
ABNORMAL	9 (42.86%)	1 (5.00%)	
NORMAL	12 (57.14%)	19 (95.00%)	
Visit 5 (Day 12)			0.067
ABNORMAL	8 (38.10%)	2 (10.00%)	
NORMAL	13 (61.90%)	18 (90.00%)	
Visit 6 (Day 14)			0.004
ABNORMAL	13 (61.90%)	3 (15.00%)	
NORMAL	8 (38.10%)	17 (85.00%)	
Visit 7 (Week 6)			0.751
ABNORMAL	9 (42.86%)	7 (35.00%)	
NORMAL	12 (57.14%)	13 (65.00%)	
Visit 8 (Week 14)			0.731
ABNORMAL	8 (40.00%)	5 (29.41%)	
NORMAL	12 (60.00%)	12 (70.59%)	
Visit 9 (Week 26)			0.166
ABNORMAL	10 (58.82%)	5 (29.41%)	
NORMAL	7 (41.18%)	12 (70.59%)	

Note: Missing values are not included in the testing

Changes in Laboratory Data

- Differences between treatments
  - Abnormal changes in RBC during treatment period

Treatment Period	BNG-1	Placebo	P-Value
NORMAL to NORMAL	5 (38.46%)	15 (78.95%)	0.030
NORMAL to ABNORMAL	8 (61.54%)	4 (21.05%)	

Vital Signs

- Heart rate and Blood pressure: no significant differences between treatments
- Decrease in body temperature in Placebo group at Visit 3
  - (Visit 1 to Visit 3: 36.74 ± 0.40°C to 36.50 ± 0.45°C, P = 0.008)

Physical Examination

- Differences between treatments:
  - Cardiovascular System at Visit 8
    - BNG-1::(10%) became abnormal, 2 stayed (10%) abnormal
    - Placebo: 0 (0%) and 0 (0%) accordingly

Brain-derived neurotrophic factors (BDNF)

- neurotrophin family.
- highest level in the cerebral cortical and hippocampal neurons.
- cultured rat hippocampal, septal and cortical neurons: protect against glutamate-induced or glucose deprivation-induced neuronal damage.

Brain-derived neurotrophic factors (BDNF)

- improve the long-term potentiation and cognitive functions after transient forebrain ischemia in rat.
- protect basal forebrain cholinergic neurons after axotomy in rat brain.
- intraventricular infusion protect the ischemia-vulnerable hippocampal CA1 neurons against ischemic injury.

Conclusion of animal study：

- Traditional Chinese medicine, BNG-1, has a protective effect on the ischemic cortical neurons against focal cerebral ischemia.
- It is possible that the protective effect of BNG-1 may act through the neurotrophic system.

Conclusion of clinical trial :

- This phase II trial involved 42 patients, 21 received BNG-1 plus aspirin and 21 received placebo plus aspirin.
- Throughout the 2 weeks of treatment and 6 months of follow-up period, the results showed BNG-1 was well tolerated by all 21 patients.
- These results support the view that BNG-1 should be further investigated in a larger sample size to evaluate its efficacy.