Phase III Clinical Trial Summary of Boluoke Capsule in Treating Ischemic Cerebrovascular Disease

Cerebral infarct is one of the most commonly seen cerebrovascular diseases; it has an abrupt onset, a high morbidity and affects people's psychosomatic health mostly. Up to date, no oral medicine with the effect of tissue-typed plasminogen activator (t-PA) has been found, though there are many drugs for treatment of cerebral infarct. Boluoke capsule is a multi-component pure-natural medicine extracted from earthworms, and includes at least two kinds of enzymes: plasminogen activator and plasmin.

Since 1990, affiliated Xuanwu Hospital of Capital Medical College, Jiangxi Provincial People's Hospital and No.2 Affiliated Hospital of Jiangxi Medical College have studied the clinical manifestations and the blood rheology changes in 453 patients with ischemic cerebrovascular disease by randomized double-blinded method.The results indicated that the total effective rate is 93.73% and the significant response rate is 73.60%. Organized by Chinese Medical Society, a collaborative group of 16 hospitals has performed a phase III clinical trial of Boluoke capsule in treating ischemic cerebrovascular disease from June 1992 to December 1993. Totally, 1560 patients were studied according to "the censorship and approval regulations of new drugs "to further verify the clinical efficacy and investigate the adverse effects. The total effective rate is 88.21% and the significant response rate is 68.91 %. Several hospitals also studied the changes in blood rheology. These results all showed that Boluoke capsule is a promising new anti-thrombotic agent with a definite efficacy and no obvious toxic/adverse effects, worthy of extensive applications.

Patients and Methods

1) Case selection: All cases in our trial were diagnosed according to the Diagnostic Criteria of the Second National Symposium on Cerebrovascular Diseases (China). They all had various degrees of hemiplegia and infarct foci shown on CT scan. The clinical efficacy of 1560 patients was investigated according to the unified therapy protocol. There were 1001 males and 559 females; the mean age was 52.38 +/- 8.13. The majority of patients took Boluoke capsule within one month from the onset (see Table 1).

2) Administration method: Two Boluoke capsules were taken orally half an hour before meal each time, three times a day for 21 days. During this period, other drugs that can dilate cerebral vessels, affect blood rheology and deoppilate the blood vessels were prohibited.

3) Appraisal of the clinical efficacy: The clinical neural deficit before and after the administration was scored by “The score criteria of clinical nervous function deficiency degree in patients with stroke” passed by the Second National Symposium on Cerebrovascular Diseases. Score 0-15 was defined mild; score 16-30 moderate and score 31-45 severe.” Beijing score criteria of clinical efficacy in patients with stroke was adopted in the finalappraisal of efficacy (score declined more than 90% was defined basically recovered; score declined from 46% to 89% significantly improved, score declined from 18% to 45% improved; score declined less than 18% or increased less than 18% no changed and deficiency score increased more than 18% deteriorated). Some hospitals also studied the changes in fibrinogen and euglobulin lysis time. The blood was sampled at 8:00-8:30AM and the sampled site was ante-cubital vein.

For more detailed information on subject selection, administration method, criteria of diagnosis and clinical efficacy, please refer to “Phase III Clinical Trial Program of Lumbrokinase in Treating Ischemic Cerebrovascular Disorders” on page 7.

4) Data processing: t-test and X^2 test were applied with TheStatistic Software of The Capital Medical College Mathematics Faculty. All data were processed by IBM-PC.

Results

The results indicated that the total effective rate was 88.21 %, and the significant response rate was 68.91 % (see Table 2). The results of those hospitals performed fibrinogen and euglobulin lysis time tests showed that fibrinogen and euglobulin lysis time were significantly reduced and the blood rheology was significantly changed after using Boluoke capsule (see Table 3 and Table 4). The adverse effects were very rare (see Table 5).

Discussion

Using earthworms as medicine was recorded in the Chinese traditional medicine masterpiece entitled ***Ben Cao Gang Mu*** *(The Catalog of Herbal Medicine).* It was deemed that the earthworm has the nature of warding off "wind" and unblocking the body's meridians and channels, and can be used to treat hemiplegia or pain syndromes due to "evil" blockages. In the 1983's international symposium of thrombosis and hemostasis, a Japanese scholar reported that the extract of earthworms had the effect of thrombolysis and denominated it "Lumbrokinase". In 1986, Korea Drugs Administration Bureau approved the manufacture of Lumbrokinase. Since then this enzyme has come into the market of many countries abroad and has been applied extensively. The trade name of Lumbrokinase in Hong Kong is "the Hart of Dragon". Boluoke capsule is a multi-component enzyme preparation and has the analogous component of t-PA, which can lyse the thrombi in vivo. There are at least two mechanisms: one is that Boluoke has the effect of plaminogen activator and the other is that it has special affinity to fibrin and thus degrades fibrin rapidly.

Because Boluoke capsule is an enzyme preparation, it is best taken half an hour before meals in order to reduce the effect of gastric acid. The low gastric volume and gastric acid enables the enzyme to be transported to intestinal tract as soon as possible.

In recent years, with further study on bioengineering technology, how large molecules such as protein penetrates the biomembrane has been extensively investigated. Fifteen years ago, Dr. Adiwl in US suggested that protein could be absorbed in vivo as peptides. In 1989, a Japanese professor verified this point by experiment. Many studies have found the general phenomena of trans-membrane transportation of protein and have elucidated theoretically that protein and enzyme can be transported transmembranely to all parts of the body and exert their normal physiological functions. Biophysics Institute of Chinese Academy of Sciences has made animal models to verify the thrombolysis effect of Boluoke. ^{125}I-Labelled fibrinogen was intermingled when the thrombi were formed on the rabbit model for acute pulmonary artery embolism. Then Boluoke was given via duodenum and radioactive intensity was measured at the time of half-an hour, 1 hour, 2 hours and 5 hours post-administration. The result demonstrated that the radioactivity significantly increased 3 hours and 5 hours after administration, which means the effect of thrombolysis was significant 4-8 hours after administration. A Japanese professor also reported that the replicated venous thrombi of Beagle dog were dissolved overtly after oral administration of Lumbrokinase.

General Information of the Patients
Table 1

	Hospital	No. of Patients			Age	Condition of the Illness			Dur. of Illness Bef.	Pretreatment
		Total	Male	Female		Mild	Moderate	Severe	Trtmt (# of Days)	Score
1	Beijing Xuanwu Hospital	210	126	84	61.51 ± 2.54	110	85	15	2.32 ± 2.54	17.16 ± 8.23
2	No. 2 Affiliated Hospital of Jiangxi Medical College	171	112	59	63.3 ± 9.13	89	73	9	3.61 ± 3.17	16.51 ± 7.11
3	Jiangxi People's Hospital	130	87	43	64.35 ± 9.83	105	20	5	26.72 ± 78.22	10.42 ± 7.50
4	Zhongshan Affiliated Hospital of Shanghai Medical College	50	27	23	66.49 ± 8.49	24	23	3	31.35 ± 37.59	15.51 ± 7.42
5	Affiliated Hospital of Tianjin Medical College	33	26	7	61.73 ± 9.38	10	17	6	26.72 ± 78.22	19.15 ± 11.36
6	Affiliated Hospital of West China Medical College	60	40	20	60.14 ± 7.78	28	27	5	6.10 ± 6.70	18.20 ± 9.15
7	Nanjing Brain Hospital	43	29	14	63.4 ± 8.78	33	7	3	13.74 ± 21.27	8.84 ± 9.75
8	Sichuan People's Hospital	50	39	11	66.2 ± 9.99	26	21	3	9.74 ± 23.76	16.2 ± 8.86
9	Affiliated Hospital of Chongqing Medical College	42	25	17	63.91 ± 9.29	29	12	1	6.21 ± 6.60	10.38 ± 8.77
10	No. 731 Hospital of Space Ministry	183	124	59	58.3 ± 9.52	120	44	19	7.23 ± 28.72	15.73 ± 8.89
11	No. 1 Handan Hospital	128	77	51	59.41 ± 8.67	95	31	2	2.72 ± 3.03	12.16 ± 7.35
12	Affiliated Hospital of Handan Second Medical School	97	53	44	62.00 ± 6.50	48	48	1	1.83 ± 2.17	16.67 ± 7.77
13	Handan Central Hospital	83	55	28	59.10 ± 8.26	46	33	4	3.33 ± 5.94	16.23 ± 8.14
14	Central Hospital of Handan Mineral Bureau	48	30	18	64.15 ± 5.28	28	20	0	1.50 ± 1.01	15.25 ± 7.13
15	An'yang People's Hospital	132	87	45	50.26 ± 8.07	80	42	10	2.97 ± 3.77	15.04 ± 9.87
16	Tangshan Workers' Hospital	100	64	36	61.31 ± 8.04	68	26	6	8.92 ± 10.15	13.54 ± 8.97
	Total	1560	1001	559	62.38 ± 8.13	939	529	92	9.93 ± 14.20	13.59 ± 8.20

Clinical Response to Boluoke® (1560 cases total)
Table 2

	Hospitals	# of Patients	Basically Recovered	Significantly Improved	Improved	No Change	Significant Response Rate	Total Effective Rate
1	Beijing Xuanwu Hospital	**210**	28 (13.33%)	98 (46.67%)	44 (20.95%)	40 (19.05%)	126 (60%)	**170 (80.95%)**
2	No. 2 Affiliated Hospital of Jiangxi Medical College	**171**	64 (37.43%)	59 (34.50%)	37 (21.64%)	11 (6.43)	123 (71.93%)	**160 (93.57%)**
3	Jiangxi People's Hospital	**130**	17 (13.08%)	51 (39.23%)	42 (32.31%)	20 (15.38%)	68 (52.31%)	**110 (84.62%)**
4	Zhongshan Affiliated Hospital of Shanghai Medical College	**50**	2 (4.00%)	16 (32%)	25 (50%)	7 (14%)	18 (36%)	**43 (86%)**
5	Affiliated Hospital of Tianjin Medical College	**33**	4 (12.12%)	14 (42.42%)	10 (30.3%)	5 (15.15%)	18 (54.55%)	**28 (84.85%)**
6	Affiliated Hospital of W. China Medical College	**60**	8 (13.33%)	25 (41.67%)	15 (25%)	12 (20%)	33 (55%)	**48 (80%)**
7	Nanjing Brain Hospital	**43**	21 (48.84%)	12 (27.91%)	4 (9.3%)	6 (13.95%)	33 (76.74%)	**37 (86.05%)**
8	Sichuan People's Hospital	**50**	4 (8%)	34 (68%)	9 (18%)	3 (6%)	38 (76%)	**47 (94%)**
9	Affiliated Hospital of Chongqing Medical College	**42**	18 (42.86%)	13 (30.95%)	4 (9.52%)	7 (16.67%)	31 (73.81%)	**35 (83.33%)**
10	No. 731 Hospital of Space Ministry	**183**	87 (47.54%)	76 (41.53%)	7 (3.83%)	13 (7.1%)	163 (89.07%)	**170 (92.9%)**
11	No. 1 Handan Hospital	**128**	19 (14.84%)	73 (57.03%)	20 (15.63%)	16 (12.5%)	92 (71.88%)	**112 (87.5%)**
12	Affiliated Hospital of Handan 2nd Medical School	**97**	22 (22.68%)	44 (45.36%)	23 (23.71%)	8 (8.25%)	67 (69.07%)	**89 (91.75%)**
13	Handan Central Hospital	**83**	26 (31.33%)	41 (49.40%)	8 (9.64%)	8 (9.64%)	67 (80.72%)	**75 (90.36%)**
14	Central Hospital of Handan Mineral Bureau	**48**	11 (22.92%)	19 (39.58%)	11 (22.92%)	7 (14.58%)	30 (62.5%)	**41 (85.42%)**
15	An'yang People's Hospital	**132**	29 (21.97%)	71 (53.79%)	21 (15.91%)	11 (8.33%)	100 (75.76%)	**121 (91.67%)**
16	Tangshan Workers' Hospital	**100**	21 (21%)	48 (48%)	21 (21%)	10 (10%)	68 (68%)	**90 (90%)**
17	Total	**1560**	381 (24.42%)	694 (44.49%)	301 (19.29%)	184 (11.79%)	1075 (68.91%)	**1376 (88.21%)**

No. of patients that have deteriorated or died during the trial: 0% out of the 1560 cases

Fibrinogen and Euglobulin Lysis Time Changes
Table 3

	Hospital [§ P<0.05 , * P<0.01]	No. of Pts	Fibrinogen Level (mg/dl)		Euglobulin Lysis Time (Minute)	
			Before Treatment	**After Treatment**	Before Treatment	**After Treatment**
1	Beijing Xuanwu Hospital	**210**	355.86 +/- 55.98	**294.77 +/- 45.15 ***	196.89 +/- 70.24	**153.40 +/- 48.00***
2	Jiangxi People's Hospital	**130**	414.39 +/- 159.15	**356.50 +/- 103.88***	242.55 +/- 64.56	**207.85 +/- 56.54***
3	Zhongshan Affiliated Hospital of Shanghai Medical College	**50**	352.14 +/- 307.31	**208.38 +/- 138.14***	N/A	N/A
4	Affiliated Hospital of Chongqing Medical College	**42**	N/A	N/A	176. 15 +/- 40.58	**129.23 +/- 59.51§**
5	No. 731 Hospital of Space Ministry	**183**	448.47 +/- 124.91	**381.56 +/- 123.35***	194.16 +/- 71.80	**156.83 +/- 55.36***
6	No. 1 Handan Hospital	**128**	304.03 +/- 39.55	**295.78 +/- 34.35***	138.08 +/- 34.49	**119.06 +/- 24.40***
7	Affiliated Hospital of Handan Second Medical School	**97**	391.54 +/- 70.66	**311.71 +/- 61.34***	152.80 +/- 33.73	**130.30 +/- 23.81***
8	Handan Central Hospital	**83**	376.87 +/- 56.32	**323.48 +/- 58.91***	151.93 +/- 44.46	**131.37 +/- 32.95***
9	Central Hospital of Handan Mineral Bureau	**48**	358.75 +/- 82.42	**314.79 +/- 50.74***	165.81 +/- 33.99	**130.46 +/- 24.53***
10	An'yang People's Hospital	**132**	390.26 +/- 54.95	**334.25 +/- 39.67***	224.11 +/- 41.57	**214.95 +/- 42.04***
11	Tangshan Workers' Hospital	**100**	330.05 +/- 83.28	**269.88 +/- 58.89***	N/A	N/A

Blood Viscosity, Plasma Viscosity, Platelet Aggregation before and after Treatment
Table 4

	Hospital (*P<0.01, § P<0.05)	# of Pts	Whole Blood Viscosity		Plasma Viscosity		PAgT	
			Pretrtmt	After Trtmt	Pretrtmt	After Trtmt	Pretrtmt	After Trtmt
1	Beijing Xuanwu Hospital	**210**	5.60 +/- 0.59	5.18 +/- 0.54*	1.83 +/- 0.12	1.72 +/- 0.09*	67.08 +/- 17.52	46.20 +/- 14.00
2	Zhongshan Affiliated Hospital of Shanghai Medical College	**50**	7.28 +/- 1.64	6.48 +/- 1.47*	1.71 +/- 0.15	1.56 +/- 0.22*	N/A	N/A
3	Nanjing Brain Hospital	**43**	7.68 +/- 2.39	6.98 +/- 1.85 §	1.90 +/- 0.80	1.85 +/- 0.58 §	N/A	N/A
4	No. 721 Hospital of Space Ministry	**183**	9.35 +/- 2.51	8.30 +/- 1.72*	1.77 +/- 0.21	11.67 +/- 0.12*	61.14 +/- 18.12	51.70 +/- 18.89
5	No. 1 Handan Hospital	**128**	8.95 +/- 1.13	8.15 +/- 0.77*	1.81 +/- 0.11	1.70 +/- 0.08*	67.89 +/- 6.72	63.63 +/- 8.65
6	Central Hospital of Handan Mineral Bureau	**48**	6.77 +/- 0.87	6.20 +/- 0.72*	1.85 +/- 0.12	1.66 +/- 0.11*	71.25 +/- 11.33	65.06 +/- 4.75
7	Affiliated Hospital of Handan Secondary Medical School	**97**	7.02 +/- 1.27	6.02 +/- 1.21*	1.83 +/- 0.12	1.70 +/- 0.10*	70.01 +/- 7.00	64.98 +/- 5.78
8	Handan Central Hospital	**83**	7.80 +/- 1.65	6.92 +/- 1.45*	1.87 +/- 0.12	1.72 +/- 0.13*	67.11 +/- 9.59	61.28 +/- 5.2
9	An'yang People's Hospital	**132**	8.27 +/- 1.73	8.19 +/- 1.26*	1.70 +/- 0.11	1.57 +/- 0.15*	57.49 +/- 22.18	47.58 +/- 22.1
#	Tangshan Workers' Hospital	**100**	4.42 +/- 0.56	4.03 +/- 0.41*	1.69 +/- 0.14	1.00 +/- 0.09*	73.08 +/- 13.39	60.10 +/- 10.

Adverse Reaction Rates (out of 1560 cases)
Table 5

Symptoms	No. of Cases	Percentage
Skin Itching	**9**	**0.60%**
Skin Rash	**3**	**0.20%**
Nausea	**18**	**1.20%**
Out of 1,560 cases: Total Cases of Adverse Reactions	**30**	**1.86%**

Phase III Clinical Trial Program of Lumbrokinase in Treating Ischemic Cerebrovascular Disorders

Objectives: Once new medication is approved for trial production by the Health Department, Phase III clinical trial proceeds. The medication will be evaluated and inspected at the society level in order to assess any adverse effect from long-term use and further study its clinical efficacy.

1. Criteria for Subjects Selection

1) Subjects with cerebral infarction from "internal carotid arterial system" were selected. Patients with cerebrovascular infarction due to posterior cerebral artery and vertebrobasilar arterial system were not included.

2) No history of residual functional impairment from stroke or other complications (including myocardial infarction, heart failure, atrial fibrillation, frequent premature contraction >15 times/minute, pneumonia, pulmonary edema, renal function impairment, gastrointestinal hemorrhage, diabetes with blood glucose >200mg%, dementia, and pseudobulbar palsy).

3) Age older than 45 years old, no upper limit.

4) Severity Criteria: According to the standards of Yangzhou Conference of Neurological Deficits Scale in Stroke, standards for level of severity is divided into: mild (score between 0-15), moderate (16-30), severe (31-45). Please refer to appendix 1-1 and 1-2.

2. Diagnostic Criteria

The main diagnostic methods are clinical signs/ symptoms and finding in CT scan. CT scan was performed upon arrival at the hospital, and cerebral hemorrhage and other conditions were excluded. If onset of disease is too early and result is inconclusive, CT scan can be repeated after 24 hours.

3. Routine Examination

1) Cranial CT

2) Blood, urine, stool routine analysis, platelet counts, bleeding time, liver function panel (GPT, TTT), renal function (BUN), prothrombin time and activity.

3) Some hospitals (if with appropriate equipment/testing condition) also test for: whole blood viscosity, plasma viscosity, hematocrit, whole blood reduced viscosity, fibrinogen, euglobulin lysis time, and PAgT.

2) and 3) are tested both before and after treatment.

4. Treatment

1) Treatment should be performed as soon as possible.

2) Oral lumbrokinase capsule, 2 capsules 3 times a day. 21 days as one treatment course.

3) For patients with massive cerebrovascular infarction and signs of cerebral hernia due to increased cranial pressure, or for patients with significant decreased level of consciousness (such as drowsiness, comatose), mannitol can be used. For patients with massive cerebrovascular infarction but their general condition is favorable, mannitol is not necessary. If blood pressure is higher than 180/100mmHg, anti-hypertensive medication may be used.

4) Balanced water intake, nutrition, electrolytes and treatment for complications can be given. Vitamin B, C and Naofukang antibiotics may also be prescribed.

5) In order to improve the correlation between the result and the efficacy of lumbrokinase, no other treatment, such as common Western and Chinese medicine for treating cerebrovascular disease, hormones, acupuncture, or massage is allowed.

5. Efficacy Criteria

1) Efficacy Criteria Standards: Criteria is set according to the "Neurological Deficits Scale in Stroke" approved in Yangzhou Conference (appendix 1) and "Criteria for Clinical Efficacy of Treatment in Stroke" (appendix 2)

2) Evaluation is performed before and after treatment.

3) Death that occurs during the course of treatment (within 3 weeks) should be included into the statistics, and reasons for subject withdrawal should be stated.

4) Cerebral hernia, pneumonia and other complications that occur during the course of treatment should be included into the statistics and the summary.

5) Final evaluation is performed at the end of the 3^{rd} week to assess efficacy and adverse reaction.

6) Analysis is performed by microcomputer statistics. Self-contrast before and after are examined by t-test and x^2 test

Appendix 1

Neurological Deficits Scale in Stroke

1. Level of Awareness	**Score**
1) Two Questions: (1) Name; (2) Month of the current date? (one month more or less can also be considered as correct)	
Both are correct	0
One is correct	1
None is correct. The following tests should be performed:	
2) Two actions (demonstration allowed)	
(1) Make a fist, release the fist and extended the fingers.	
(2) Close the eyes, open the eyes.	
Both are completed	3
One can be completed	4
None can be completed. The following tests should be performed:	
3) Strong Local Stimulation (on the unaffected side)	
Avoidance Movement	6
Limb retraction	7
Limb stretched	8
Non-responsive	9
2. Function of Horizontal Gaze	
Normal	0
Limited lateral gaze	2
Horizontal gaze nystagmus	4
3. Facial Paralysis	
Normal	0
Mild paralysis, able to move to some extent	1
Complete paralysis	2

4. Speech

Normal	0
Able to communicate but with difficulty in expressing	2
Able to perform simple communication	3
Able to communicate with single vocabulary, using facial and body language as assistance	5
Unable to Communicate	6

5. Arm and Shoulder Muscle Strength

Normal V◦	0
IV◦	1
III◦ able to abduct arm to the level higher than shoulder	2
III◦ able to abduct arm to the level at or lower than shoulder	3
II◦	4
I◦	5
0◦	6

6. Hand Muscle Strength

Normal V◦	0
IV◦	1
IV◦ able to make a fist, release the fist and stretch fingers	2
III◦ able to bend but not stretch fingers	3
II◦ able to bend fingers but finger tips can't reach the palm	4
I◦ able to move fingers but with very limited range of motion	5
0◦	6

7. Lower Extremity Muscle Strength

Normal V◦	0
IV◦	1
III◦ Able to elevate leg to >45◦, able to move ankle and toes	2
III◦ Able to elevate leg to about 45◦, unable to move ankle or toes	3
II◦ able to elevated leg above the bed, but < 45◦	4
I◦ able to move leg in horizontal plane, unable to elevate	5
0◦	6

8. Ability to Walk

Normal	0
Able to walk independently for 5 meter, with limping	1
Able to walk independently with crutch	2
Able to walk with assistance	3
Able to stand, but unable to walk	4
Able to sit, but unable to stand	5
Bedridden	6

Highest score: 45. Lowest score: 0.

Mild: 0-15
Moderate: 16-30
Severe: 31-45
(Modified Edinburgh-Scandinavia Research Group)

Appendix 2

Criteria for Clinical Efficacy of Treatment in Stroke

Clinical efficacy assessment is based on:

Percentage of accumulative score from Neurological Deficits Scale (functional improvement), and the result is processed by statistical methods (x^2, p value)

Cured: Neurological Deficits Scale score decreased by 90%.
Significantly Improved: Neurological Deficits Scale score decreased by 46-89%.
Improved: Neurological Deficits Scale score decreased by 18-45%.
No Change: Neurological Deficits Scale score decreased or increased by less than 18%.
Exacerbation: Neurological Deficits Scale score increased by 18% or more.
Death