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ORIGINAL INVESTIGATION

Effects of Batroxobin with Continuous Transcranial Doppler Monitoring in Patients with Acute Cerebral Stroke: A Randomized Controlled Trial

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Our objective was to determine whether continuous transcranial Doppler (TCD) monitoring could safely enhance the efficacy of batroxobin, a thrombin-like enzyme extracted from *Bothrops atrox moojeni* venom, in the treatment for acute cerebral stroke beyond the thrombolytic time window. Ninety patients suffering an acute cerebral stroke were recruited into the study within 12 hours after the onset of symptoms. Patients were randomized to receive batroxobin with (target group) or without 1 hour of continuous TCD monitoring (control group). Clinical evaluation of stroke was based on the National Institutes of Health Stroke Scale (NIHSS) score, Barthel index (BI), Thrombolysis in Brain Ischemia score (TIBI), the incidence of advancing stroke, and the recurrence of cerebral infarction. The patients receiving continuous TCD monitoring showed significant improvement in NIHSS score at 57 days post treatment compared with the control. Similarly, patients receiving continuous TCD monitoring also showed significant improvement in BI at 3 months compared with the controls. Consistently, both the incidence of advancing stroke after 1 week and the incidence of stroke recurrence after 3 months were significantly lower in TCD monitored group than control group. Moreover, the safety of the employment of TCD monitoring in the treatment of these patients was confirmed as there was no significant difference of the incidence of intracranial hemorrhage at 1 week after the treatment between the target and control groups. Taken together, our study showed that batroxobin, in combination with continuous TCD monitoring at the middle cerebral artery, reduced the incidence of advancing stroke and stroke recurrence after treatment without adverse effects in terms of poststroke intracranial hemorrhage. (Echocardiography 2014;00:1–10)

Key words: transcranial Doppler, batroxobin, cerebral stroke, advancing stroke

Ischemic cerebral stroke remains one of the leading causes of death worldwide and continues to impose a considerable long-term economic burden and health services for patients.[1] Currently, the only FDA approved drug for the treatment of acute ischemic stroke is tissue plasminogen activator (tPA), which is administered intravenously within 3 hours following the onset of symptoms. Unfortunately, the therapeutic effects are far from satisfaction. Only 30–40% of treated patients are able to achieve early recanalization. Therefore, new therapeutic strategies are needed to increase the rate of arterial recanalization in patients within and beyond the 3-hour tPA optimal treatment window.

Ultrasound exposure can generate bioeffects through cavitation mechanical[2] and/or thermal mechanisms,[3–5] which have been shown to increase the penetration of tPA into thrombus[6] and reversibly disaggregate fibrin structure to improve the binding of tPA to fibrin. Sonothrombolysis therapy, which involves the administration of tPA in combination with transcranial Doppler (TCD), has been tested in the treatment of acute cerebral stroke in both animal experiments[7–12] and clinical studies.[13–18] Results demonstrated that sonothrombolysis therapy significantly improved recanalization and neurological function. Moreover, unlike traditional therapeutic ultrasound apparatuses, TCD has the advantage of real time monitoring with

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accurate positioning. Because of the small time window for administration of tPA, less than 5% of patients in the United States who experienced acute ischemic stroke have been able to receive combined tPA and TCD treatment, thus leaving a considerable proportion of patients who may not achieve arterial recanalization. These numbers are even less encouraging in other countries. In China, the success rate for thrombolytic therapy in the treatment of acute cerebral stroke is even lower than 1%.

Currently, alternative therapeutic strategies are being evaluated in the clinic. One promising therapy involves the use of batroxobin, a thrombin-like enzyme extracted from *Bothrops atrox moojeni* venom with unique antiinflammatory, anticoagulation, and plasma fibrinogen degradation properties. It has been proven clinically that batroxobin can attenuate pathophysiological changes in acute cerebral stroke patients by affecting various targets. The goal of our study was to determine whether continuous TCD monitoring can safely enhance the efficacy of batroxobin in the treatment of patients with acute cerebral stroke beyond the 3-hour thrombolytic time window.

Material and Methods:

Subjects:

Ninety patients with an acute cerebral stroke in the M1 section of middle cerebral artery were enrolled in this study within 12 hours after the onset of symptoms. Approval was obtained from the international review board (IRB) at Shenzhen People's Hospital. Written informed consent for participation in the trial was provided by all participants or their legal representatives.

Inclusion Criteria:

(1) Age <70 years. (2) Patients who had acute cerebral thrombosis (corresponding vessel was M1 section of middle cerebral artery). (3) Patients who had acute cerebral thrombosis over 3 hours but less than 12 hours after onset of the symptoms or within 3 hours after onset of the symptoms but not eligible for thrombolysis treatment. (4) Definite paralysis of limbs (muscular strength from 0 to 4 grades). (5) Noncomatose patients with cerebral stroke.

Exclusion Criteria:

(1) Age ≥70 years. (2) Vertebral basilar system stroke and cerebral hemorrhage. (3) Exanimation. (4) Lacunar stroke without obvious limbs dyskinesia. (5) Combination of life-threatening complication. (6) Having received thrombolysis treatment. (7) Obvious hemorrhagic tendency or blood platelets count (BPC) $<80 \times 10^9$/L. (8) Having received plasminogen drug or snake venom preparations. (9) Patients with severe heart, lung, and kidney functional disturbance or psychosis. (10) Pregnant or lactating women. (11) Unclear temporal window. (12) Participating in other clinical trials.

Study Protocol:

Eligible patients were randomly divided into control and target groups using a random digits table. Both groups received a single dose of 10 units of batroxobin (TOBASHI, Beijing, China) by intravenous infusion. Simultaneously, the target group received 1-hour TCD continuous monitoring at the occluded M1 section of middle cerebral artery, whereas the control group received 1-hour placebo monitoring. Both groups were also treated with aspirin (0.1 g/day) and atorvastatin calcium (20 mg/day). TCD monitoring was performed with a TCD cerebral vascular diagnostic/monitoring system and an automatic custody probe (EMS-9UA*2P, Shenzhen Delica Electronics Co., Ltd., Shenzhen, Guang Dong Province, China) with the capability of synchronous multidepth custody at the middle cerebral artery. Monitoring was performed at the depth of 55–60 mm for M1 middle cerebral artery occlusion. The operating frequency was 2 MHz, and the output energy was set at 0.35 W.

Observation Index:

All the patients underwent cranium CT examination before enrollment to exclude patients with intracranial hemorrhage. Middle cerebral artery occlusion was confirmed by conventional TCD. During the trial, cranium CT examination was implemented immediately for patients with symptoms of clinical deterioration and objective signs. All patients were reexamined by cranium CT scan 1 week after treatment. Blood flow rate (BFR) in the occluded middle cerebral artery was serially examined by TCD at 1, 3, 5, 7 days, and 3 months after batroxobin treatment. Thrombolysis in Brain Ischemia (TIBI)[19] scale was recorded according to the BFR.

Blood was carefully drawn from each patient and levels of white blood cells (WBC), ant thrombin III, protein C, homocysteic acid, supersensitive C reactive protein (SCRP), and D dimerade were measured before treatment, as well as 7 days and 3 months after treatment. Furthermore, fibrinogen was measured before treatment and also at 3 days and 3 months after treatment. Renal function and liver function were examined before treatment and at 1 week after treatment.

Clinical Evaluation:

Stroke severity was assessed by National Institutes of Health Stroke Scale (NIHSS) score before bat-

roxobin treatment and at 1, 3, 5, 7 days, and 3 months after treatment. Functional disability was measured by Barthel index (BI) before treatment and 3 months after treatment. Incidence of advancing stroke and intracranial hemorrhage 1 week after treatment and stroke recurrence 3 months after treatment were also recorded. NIHSS and BI represent the neurologic functional recovery in early and long term, respectively. Incidence of advancing stroke and stroke recurrence represents the effect of the treatment on the ischemic stroke in early term progress and long-term recurrence, respectively. Intracranial hemorrhage represents the safety of the treatment.

Statistics Analysis:
Data analyses were performed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). A value of $P < 0.05$ was accepted as statistically significant. Data were described as mean $\pm$ standardized deviation. Ranked data were described as median. Rank-sum test and *t*-test were applied in analyses according to the dispersion pattern of date.

Result:

Subjects:
A total of 90 patients with acute cerebral stroke were enrolled according to the inclusion criteria in this trial at the department of neurology, Shenzhen People's hospital from September 2010 to June 2011. Forty-seven subjects (6 females, 41 males) were randomly assigned to the control group, and 43 (8 females, 35 males) were assigned to the target group. Baseline characteristics showed no difference between the target group and the control group (Table I; Fig. 1).

TABLE I

Baseline Characteristics of Subjects

	Control Group (N = 47)	Target Group (N = 43)	P Value
Mean age (year)	55.72 ± 9.84	58.91 ± 11.64	0.165
Mean time from onset of symptoms to batroxobin treatment (hour)	9.70 ± 2.77	9.23 ± 2.35	0.390
NIHSS score	6.72 ± 3.03	7.33 ± 3.27	0.370
Barthel index	50.43 ± 18.32	51.98 ± 19.58	0.699
WBC (10^9/L)	8.57 ± 2.86	8.53 ± 2.28	0.928
Fibrinogen (g/L)	2.97 ± 0.91	3.12 ± 0.75	0.393
Antithrombin III	100.62 ± 13.50	99.39 ± 13.84	0.673
SCRP (mg/L)	4.74 ± 5.99	4.26 ± 5.36	0.693
Protein C (%)	108.44 ± 18.85	105.51 ± 22.78	0.680
TIBI grade median	4	4	0.942
Case of complete follow-up	41	36	0.636

NIHSS = National Institutes of Health Stroke Scale; WBC = white blood cells; SCRP = supersensitive C-reactive protein; TIBI = thrombolysis in brain ischemia.

Clinical Outcomes:
Patients in the target and control groups had similar baseline of stroke severity as measured by NIHSS (Table II). At earlier time points after treatment, no significantly difference of NIHSS was observed between the two groups including at 24 h (Fig. 2) and 3 days (Fig. 3) ($P = 0.464$, 0.163). However, at 7 days after treatment (Fig. 4), both groups showed improvements in NIHSS scores compared with those before treatment (4.24 ± 3.37 vs. 6.72 ± 3.03 in the control group, 2.23 ± 2.48 vs. 7.33 ± 3.27 in the target group). Particularly, at 5 days (Fig. 5) and 7 days (Fig. 4) after treatment, patients receiving continuous TCD monitoring had greater improvement in NIHSS scores than patients in control group (2.91 ± 2.78 vs. 4.36 ± 3.42 at 5 days, $P = 0.030$, and 2.23 ± 2.48 vs. 4.24 ± 3.37 at 7 days, $P = 0.002$).

Before receiving treatment, there is also no difference of functional disability as indicated by baseline BI in both target (Fig. 1) and control groups (Table III). After 3 months following batroxobin treatment, both groups showed significant improvement in BI compared to the scores before treatment (Fig. 6). Similar to NIHSS, the increase of BI was also more in the target group and patients in this group showed a significantly higher rate of favorable functional outcome compared with those in the control group ($P = 0.000$).

Although plasma fibrinogen in the target group was significantly lower than that in the control group at 3 days after treatment (1.82 ± 0.48 vs. 2.10 ± 0.73 g/L, $P = 0.033$), the difference was not significant at 3 months after treatment, even though the levels of plasma fibrinogen were slightly lower in the target group when compared with the control group.

In patients receiving TCD monitoring, a slight, but nonsignificant increase in plasma C and antithrombin III levels was observed at 1 week and 3 months after treatment when compared to the controls (Table IV). The plasma WBC of the target group increased at 1 week after treatment and decreased at 3 months after treatment compared with the control group, but failed to prove significant. A similar trend was observed in the levels of SCRP at 1 week or 3 months after treatment.

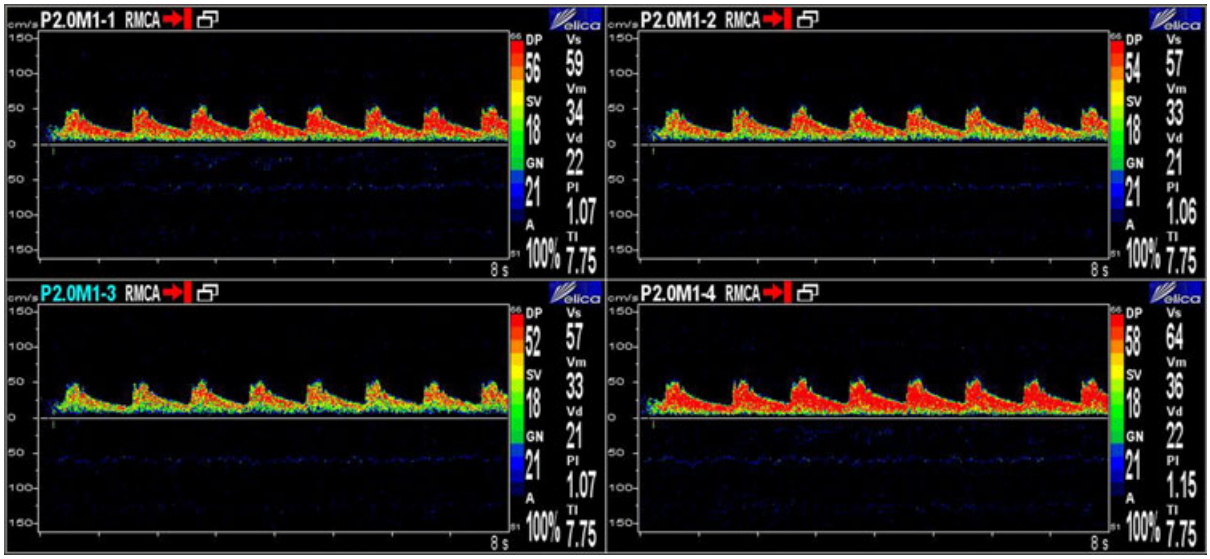

Figure 1. TCD images from right MCA were obtained at depth of 52–58 mm, at 10 hours after the onset of symptoms while the patient were receiving a single dose of 10 units of batroxobin by intravenous infusion. The mean blood flow velocity (Vm) was 33–36 cm/sec. Peak systolic blood velocity (Vs) was 57–64 cm/sec. End-diastolic blood flow velocity (Vd) was 21–22 cm/sec. TIBI grade was 3. NIHSS score was 15. BI score was 45.

TABLE II

Comparison of NIHSS Score before and after Treatment

Time	Control Group (N = 47)	Target Group (N = 43)	t-Value	P-Value
Before treatment	6.72 ± 3.03	7.33 ± 3.27	0.907	0.367
24 hour after treatment	6.04 ± 4.00	5.47 ± 3.39	0.735	0.464
3 days after treatment	5.06 ± 3.66	4.05 ± 3.16	1.406	0.163
5 days after treatment	4.36 ± 3.42	2.91 ± 2.78	2.205	0.030
7 days after treatment	4.24 ± 3.37*	2.23 ± 2.48†	3.212	0.002

NIHSS = National Institutes of Health Stroke Scale.
*NIHSS score 7 days after treatment in control group was significantly higher than that before treatment (P = 0.000).
†NIHSS score 7 days after treatment in target group was significantly higher than that before treatment (P = 0.000).

Both batroxobin and its combination with TCD treatment significantly enhanced recanalization from similar baselines (P = 0.942) as characterized by the TIBI score at 1 week and 3 months after treatment, respectively (P = 0.000, 0.001, 0.000, 0.000) (Table V). However, TCD monitoring help to improve batroxobin-mediated enhancement in TIBI score only at 3 months after treatment (P = 0.002).

Adverse Events:

A repeat CT scan 1 week after treatment identified the following: 1 patient with subarachnoid hemorrhage, 2 patients with intracerebral hemorrhage in the control group, and 2 patients with intracerebral hemorrhage in the target group. The occurrence of postintracranial hemorrhage showed no significant difference between the two groups (P = 1.000) (Table VI).

The incidence of advancing stroke in the target group (2.3%) was lower than that in the control group (17.0%) (P = 0.049) within 1 week after the treatment (Table VII). Moreover, the stroke recurrence in the target group (0.0%) was significantly lower than that in the control group (12.80%) (P = 0.045) at 3 months after treatment (Table VIII).

No significant difference in the concentration of serum CRE and GOT was observed between the placebo and TCD-treated groups after measuring the change in serum protein levels 7 days posttreatment (Table IX). No deaths were reported for either group.

Discussion:

Our study shows that TCD monitoring safely augments the effect of batroxobin in the treatment of patients with acute cerebral stroke. Batroxobin

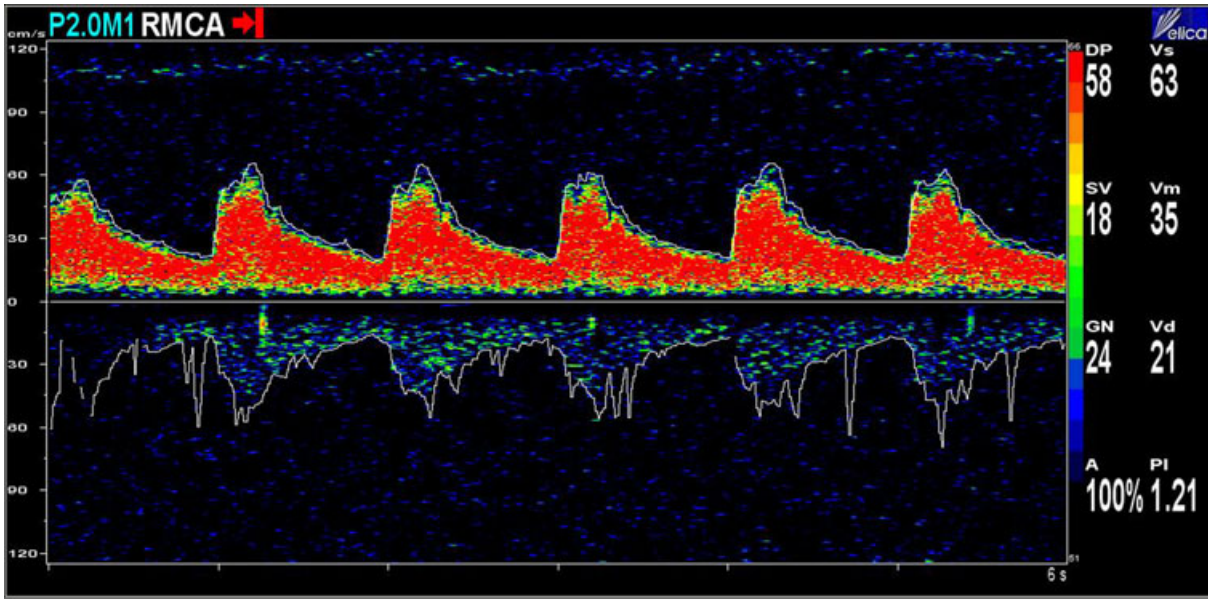

Figure 2. An image from right MCA was obtained at depth of 58 mm 24 hours after receiving the TCD–batroxobin combined therapy. Vm was 35 cm/sec. Vs was 63 cm/sec. Vd was 21 cm/sec. TIBI grade was 3. NIHSS score was 12.

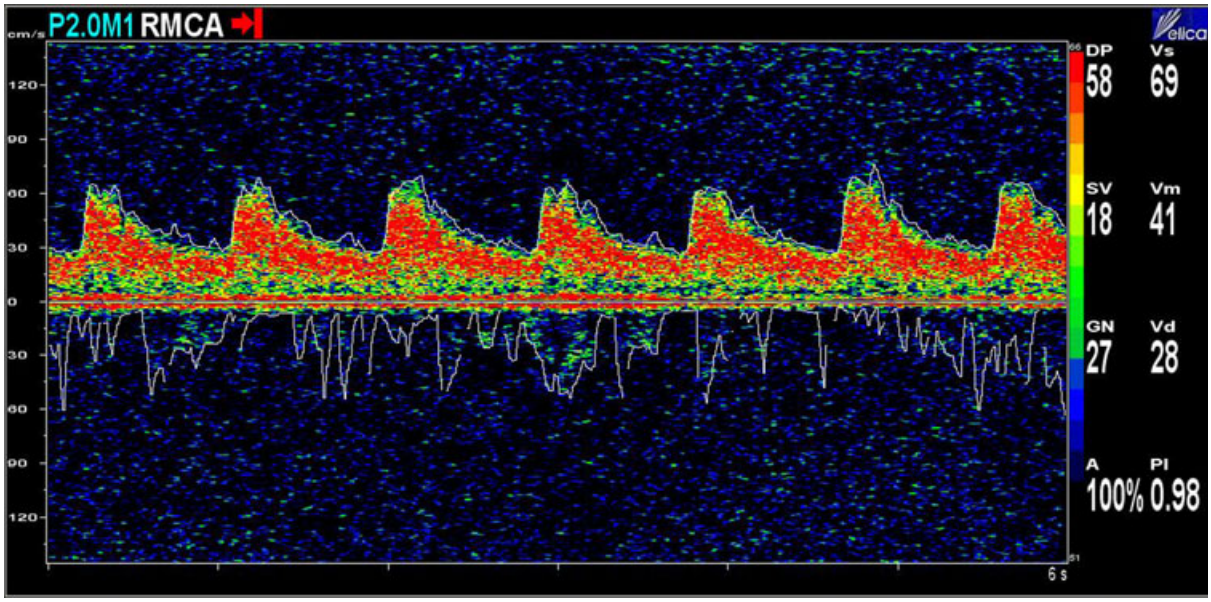

Figure 3. An image from right MCA was obtained at depth of 58 mm 3 days after receiving the TCD–batroxobin combined therapy. Vm was 41 cm/sec. Vs was 69 cm/sec. Vd was 28 cm/sec. TIBI grade was 3. NIHSS score was 12.

is a thrombin-like enzyme that has been shown to mediate plasma fibrinogen degradation, anticoagulation, thrombolysis, and antiinflammation effects, all of which have been proven to be clinically relevant when examining the pathophysiological changes in target organs for patients suffering an acute cerebral stroke. Plasma fibrinogen, an indice of a hypercoagulable state and thrombogenesis, is the main target of batroxobin.[20,21] In this study, plasma fibrinogen in our target group was significantly lower than that in our control group at 3 days after treatment. At 3 months after treatment, the target group showed a slight, but nonsignificant, reduction in plasma fibrinogen levels compared with the control group. These findings indicate that TCD

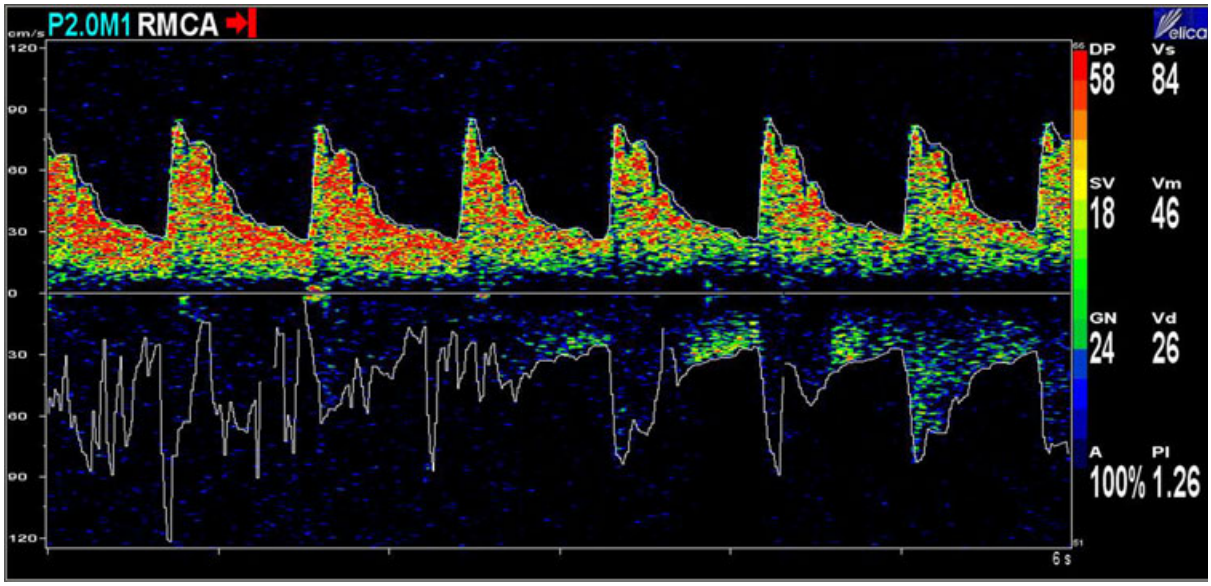

Figure 4. An image from right MCA was obtained at depth of 58 mm 7 days after receiving the TCD–batroxobin combined therapy. Vm was 46 cm/sec. Vs was 84 cm/sec. Vd was 26 cm/sec. TIBI grade was 5. NIHSS score was 6.

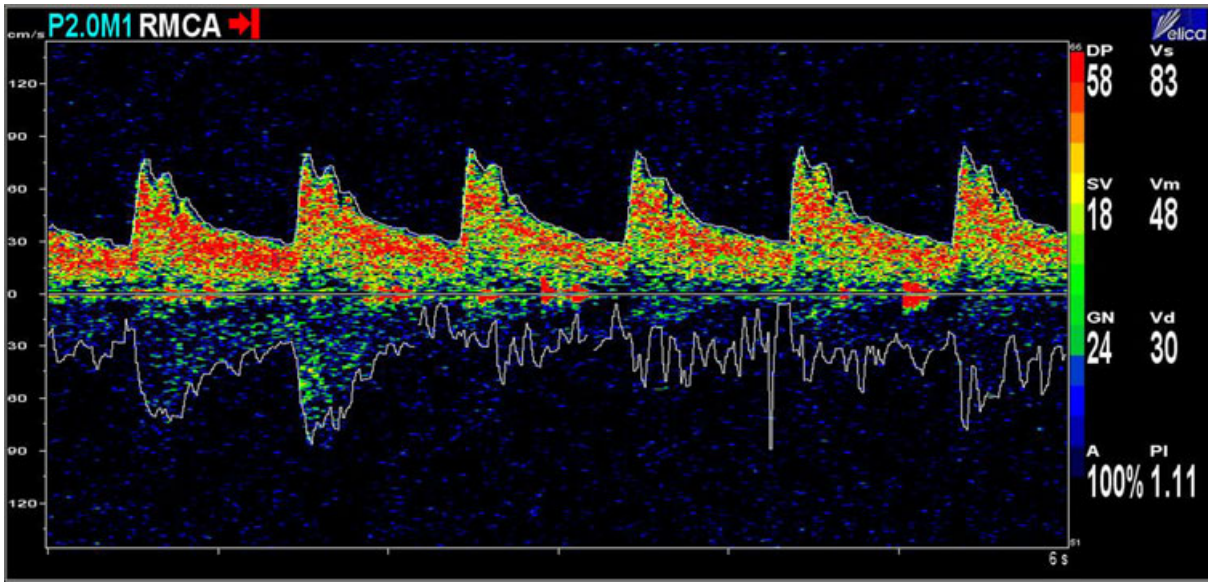

Figure 5. An image from right MCA was obtained at depth of 58 mm 5 days after receiving the TCD–batroxobin combined therapy. Vm was cm/sec. Vs was 83 cm/sec. Vd was 30 cm/sec. TIBI grade was 5. NIHSS score was 8.

accelerates the ability of batroxobin to deplete plasma fibrinogen over a short term. Several mechanisms might be involved in this process including: (1) cavitation[2] of the ultrasound causes the microbubbles in the blood to expand and contract with each other, which leads to the microstreaming of the fluids surrounding the cells.[22] When bubble oscillations are so large, the bubble collapses. Disintegration of a microbubble can form local, multiple extreme physico-conditions, such as high-pressure shock waves, increases in temperature, and high speed filament bands. These events can facilitate the penetration of batroxobin into the thrombus, breakages of the fibrin wire frame of the thrombus, exposure of the batroxobin binding site in thrombus and fibrinogen depletion. (2) Mechanical vibration induced by the ultrasound can lose the compact fibrin of

TABLE III

Comparison of Barthel Index

Time	Control Group (N = 47)	Target Group (N = 43)	t-Value	P-Value
Before treatment	50.43 ± 18.32	51.98 ± 19.58	0.388	0.699
3 months after treatment	79.57 ± 20.24*	94.40 ± 9.95†	4.421	0.000

*In control group, the difference between Barthel index before treatment and 3 months after treatment was significant (P = 0.000).
†In target group, the difference between Barthel index before treatment and 3 months after treatment was significant (P = 0.000).

the thrombus and fully expose the binding sites to batroxobin. Moreover, mechanical vibration can accelerate fluid axial flow and induce acoustic streaming, which forms a light velocity gradient on the surface of the thrombus. The shearing force induced by the light velocity gradient can mechanically destruct the surface of the thrombus and further augment the exposure of fibrinogen to batroxobin.[23] (3) Temperature elevation[3,4] caused by the thermal effect of the ultrasound might enhance the activity of batroxobin by facilitating the binding and penetration of batroxobin to the thrombus.

In this trial, we did not observe that ultrasound exposure enhanced the anticoagulatory or antiinflammatory capabilities of batroxobin as measured by plasma protein C, antithrombinIII, WBC, and SCRP levels. The possible explanations may include: (1) anticoagulation and anti-inflammation are not the main functions of batroxobin; (2) plasma WBC and SCRP levels are influenced not only by the inflammatory reaction induced by cerebral stroke but also by infection, anxiety–depression state, hypertension, diabetes, and other factors.

In both groups, TIBI grades at 1 week or 3 months after treatment were higher than those before treatment. However, compared to batroxobin treatment alone, combined therapy did not show a substantial benefit in the earlier stages of the trial. Although batroxobin and TCD together can further promote the restoration of blood flow in diseased vessel in long-term possibly due to the unique physical and chemical effects of TCD, it might also be related to several other reasons. Patients enrolled in this trail were over the thrombolysis time window. It is impossible to achieve instant thrombolysis and restore the blood flow immediately. This combined therapy was capable of the fibrinogen depletion and partial thrombolysis as a result of batroxobin treatment and the unique physical and chemical effects of TCD. Therefore, the main function of the combined therapy was to enhance the dissolving of a newly formed thrombus, repression of thrombus recontouring, and formation of collateral circulation. These activities might help determine the long-term

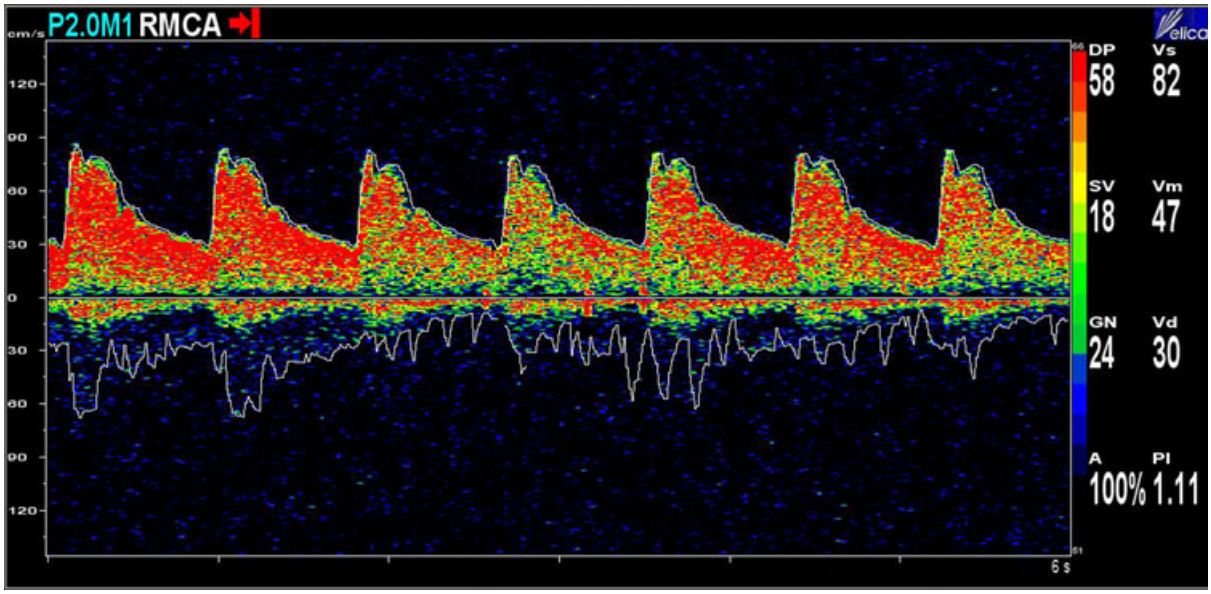

Figure 6. An image from right MCA was obtained at depth of 58 mm 3 months after receiving the TCD–batroxobin combined therapy. Vm was 46 cm/sec. Vs was 84 cm/sec. Vd was 26 cm/sec. TIBI grade was 5. NIHSS score was 2. BI score was 95 (BI is an ordinal scale used to assess neurological functional recovery in long-term therefore was not repeated in acute phase).

TABLE IV

Comparison of Blood Parameters

Blood Parameters	Control Group (N = 47)	Target Group (N = 43)	t-Value	P-Value
Plasma antithrombin III a week after treatment (%)	108.08 ± 13.79	108.65 ± 12.50	0.202	0.841
Plasma antithrombin III 3 months after treatment (%)	102.49 ± 19.61	109.10 ± 15.50	1.613	0.111
Plasma protein C (%) a week after treatment	105.79 ± 28.93	106.36 ± 25.35	0.066	0.948
Plasma protein C (%) 3 months after treatment	104.51 ± 26.88	113.00 ± 22.86	0.962	0.344
Plasma SCRP a week after treatment (mg/L)	11.48 ± 20.24	9.74 ± 15.72	0.448	0.656
Plasma SCRP 3 months after treatment (mg/L)	7.59 ± 14.59	4.63 ± 7.13	1.140	0.259
Plasma WBC a week after treatment (10^9/L)	7.89 ± 2.40	8.21 ± 2.31	0.642	0.522
Plasma WBC 3 months after treatment (10^9/L)	7.72 ± 2.93	7.10 ± 2.00	1.067	0.290
Plasma fibrinogen 3 days after treatment (g/L)	2.10 ± 0.73*	1.82 ± 0.48†	2.164	0.033
Plasma fibrinogen 3 months after treatment (g/L)	3.06 ± 1.07	2.89 ± 0.72	0.818	0.416

SCRP = supersensitive C reactive protein; WBC = white blood cells.
*In control group, there was significant difference between plasma fibrinogen before treatment and 3 days after treatment (P = 0.000).
†In target group, there was significant difference between plasma fibrinogen before treatment and 3 days after treatment (P = 0.000).

TABLE V

Comparison of TIBI Grade Median

Time	Control Group (N = 47)	Target Group (N = 43)	Z-Value	P-Value
Before treatment	4	4	−0.073	0.942
24 hours after treatment	4	4	−0.721	0.471
3 days after treatment	4	4	−0.603	0.546
5 days after treatment	4	5	−1.859	0.063
7 days after treatment	5*	5†	−1.860	0.063
3 months after treatment	4‡	5§	−3.092	0.002

TIBI = thrombolysis in brain ischemia.
*In control group, TIBI grade median 7 days after treatment was higher than that before treatment (P = 0.000).
†In target group, TIBI grade median 7 days after treatment was higher than that before treatment (P = 0.000).
‡In control group, difference between TIBI grade median 3 months after treatment and that before treatment was statistically significant (P = 0.001), whereas the difference between TIBI grade median 3 months after treatment and that 7 days after treatment was not statistically significant (P = 0.132).
§In target group, difference between TIBI grade median 3 months after treatment and that before treatment was statistically significant (P = 0.000), whereas difference between TIBI grade median 3 months after treatment and that 7 days after treatment was not statistically significant (P = 0.180).

effect of combined therapy on restoration of blood flow in the corresponding vessel.

Neurological function was measured by NIHSS and BI. We found that those patients

TABLE VI

Comparison of Intracranial Hemorrhage Ratio

	No Poststroke Hemorrhage	Poststroke Hemorrhage	χ^2 Value	P-Value
Control group (N = 47)	44 (93.6%)	3 (6.4%)	0.000	1.000
Target group (N = 43)	41 (95.3%)	2 (4.7%)		

TABLE VII

Comparison of Incidence of Advancing Stroke

	Not Occurred	Occurred	χ^2 Value	P-Value
Control group (N = 47)	39 (83.0%)	8 (17.0%)	3.879	0.049
Target group (N = 43)	42(97.7%)	1(2.3)		

TABLE VIII

Comparison of Recurrence of Cerebral Stroke 3 Months after Treatment

	Not Occurred	Occurred	χ^2 Value	P-Value
Control group (N = 47)	41 (87.2)	6 (12.8%)	4.009	0.045
Target group (N = 43)	43 (100%)	0 (0.0%)		

TABLE IX

Comparison of Serum Creatinine and Glutamic Oxalacetic Transaminase Levels

Blood Parameter	Control Group (N = 47)	Target Group (N = 43)	t-Value	P-Value
Serum CRE before treatment (μmol/L)	89.31 ± 26.98	98.12 ± 38.75	−1.240	0.219
Serum CRE a week after treatment (μmol/L)	85.01 ± 22.64	90.14 ± 41.93	−0.700	0.487
Serum GOT before treatment (U/L)	22.13 ± 8.79	22.65 ± 6.71	−0.315	0.753
Serum GOT a week after treatment (U/L)	27.18 ± 15.19	27.66 ± 10.69	−0.168	0.867

CRE = creatinine; GOT = glutamic oxalacetic transaminase.

receiving continuous TCD monitoring showed greater improvement in NIHSS at 5 days and 7 days and an improvement in BI at 3 months. The results indicate that batroxobin combined with 1 hour of continuous TCD monitoring can augment the effect of batroxobin in neurologic functional recovery over short- and long-term follow-up times. This might be due to the ability of the ultrasound to enhance the binding of batroxobin to its thrombus substrates, augment its enzymatic activity, inhibit further thrombus formation, and enhance the survival of cells in ischemic penumbra.

The target group showed a lower incidence of advancing stroke at 1 week after treatment and a lower rate of stroke recurrence at 3 months after treatment. There was no patient death in both groups within 3 months after treatment. These data indicate that the combined treatment can decrease the incidence of advancing stroke and stroke recurrence, even 3 months after treatment. The cause of the progression and stroke recurrence are associated with multiple mechanisms. Repression of thrombus formation and partial thrombolysis by combined treatment at acute state might partially explain the decrease in incident of advancing stroke and stroke recurrence in the target group. It is not clear whether TCD had a direct and beneficial effect on the blood vessel and nerve tissue. Further studies are needed to address this issue.

There was no significant difference in the incidence of poststroke hemorrhage between the two groups. According to our results, the liver and renal functions were normal in all the subjects. This indicates that the application of 1-hour continuous TCD monitoring neither increase the incidence of poststroke hemorrhage nor had any harmful effects on liver and renal function. This study provided preliminary safety data of the combined treatment. It has been reported that the thermal effects of ultrasound may cause skin burns and discomfort,[24] but we did not observe these effects in our study.

The patients enrolled in our study were diagnosed with relatively mild clinical symptoms, because patients with critical clinical symptoms poorly performed in cooperation with TCD monitoring and had multiple complications. A stratified study could be implemented in the future to gather more information about the therapeutic effects of batroxobin in combination with TCD in patients with critical clinical symptoms. Optimization of this combined treatment for acute cerebral stroke would require a larger sample size, multiple study centers, and fine tuning of the energy/frequency ratio of TCD for thrombolysis treatment.

In summary, our study showed that application of 1-hour continuous TCD monitoring to patients with acute cerebral stroke within 12 hours after the onset of the symptoms can augment the fibrinogen-depleting effect of batroxobin treatment, improve the recovery of neurological function, and decrease the incidence of advancing stroke and stroke recurrence without increasing poststroke hemorrhage.

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