Anticoagulation Therapy is Harmful to Large-Sized Cerebellar Infarction

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SUMMARY

Aim: Anticoagulants are commonly used to treat ischemic stroke. Its impact on cerebellar infarction has not been fully understood. **Methods:** In the clinical study, we reviewed a consecutive series of patients with large-sized cerebellar infarction (diameter > 3 cm, $n = 30$) treated with or without anticoagulation. In animal study, cerebellar infarction operation was performed in 12 Cynomolgus monkeys. Then the animals were administered with low molecular weight heparin (LMWH) or vehicle for 14 days. **Results:** Six patients died during the following treatment. All the subjects that died received anticoagulation therapy, while nobody in the survival group received such a therapy. Compared with sham-operated animals, all monkeys with cerebellar infarction have obvious neurological deficits. The number and size of the Purkinje cells in the cerebellar area were also reduced. Two animals in the LMWH group (33%) died, while all animals in the vehicle control group survived. Compared with the vehicle group, the neurological score in the LMWH group was significantly increased ($P < 0.05$). The water content in the cerebella was also significantly higher ($P < 0.05$). Edema, hemorrhage, and subarachnoid hemorrhage occurred in the cerebella as well as brainstem of all the LMWH treated animals. **Conclusions:** These results indicated the harmful effects of anticoagulation therapy on large-sized cerebellar infarction.

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The first two authors contributed equally to this work.

Introduction

Cerebellar infarction is a subtype of stroke. There are about 20,000 new patients with cerebellar infarction per year in the U.S. [1,2]. However, cerebellar infarction has not received the same level of attention in medical publication as the well-defined anterior circulation and brainstem stroke syndromes. This might be due to its diverse clinical presentation with uncommon and non-specific symptoms. The important neurological examination, including coordination, gait, and eye movements, may help identify cerebellar infarction. With increasing availability of magnetic resonance imaging (MRI), especially the Diffusion Weight MRI (DW MRI), the diagnosis of cerebellar infarction has become easier. Unfortunately, there are no data from randomized trials that are specific for patients with cerebellar infarction. The neurologists often manage cerebellar infarction according to the guidelines designed for the anterior circulation infarction [3], which might be potentially dangerous because of the specific anatomy of cerebellum other than cerebrum. Edema from infarction, especially

large-sized ones, in the posterior fossa, can result in potentially fatal complications, such as brainstem compression and obstructive hydrocephalus. Therefore, the management of cerebellar infarction might also prevent a second and more devastating stroke.

Anticoagulants have been used to treat acute ischemic stroke for more than 50 years. They are often prescribed to patients with recent stroke in an effort to prevent early recurrent stroke and to improve neurological outcomes. But the effectiveness of emergency anticoagulation has not been fully confirmed [4–11]. Despite the great controversy, the anticoagulants continue to be prescribed commonly [12]. Especially, a multicenter, randomized trial study recently shows that anticoagulants may be of benefit in certain subgroups of patients with cerebral infarction, including patients with posterior circulation stenosis [13]. These data seemed to support the beneficial effect of anticoagulation on posterior circulation and brainstem stroke syndromes, including cerebral infarction.

To evaluate the impact of anticoagulation therapy on the outcome of cerebellar infarction, especially the large-sized

infarction, a consecutive series of patients with severe cerebellar infarction (infarct diameter > 3 cm) treated with anticoagulation or not were reviewed, and Cynomolgus monkeys were subjected to cerebellar infarction operation to ensure the risk of anticoagulation.

Materials and Methods

Clinical Study

The clinical study reviewed a consecutive series of patients in the department of neurology, Changhai hospital, hospitalized from August, 2010 to December, 2011 with ethics committee approval. There are 30 patients (27 men and three women) with large-sized cerebellar infarction (infarct diameter > 3 cm). They were mainly administered three therapies: (1) anti-coagulation: low-molecular-weight heparin (LMWH: Fraxiparin, 4000 u, *isc*, *bid*) or argatroban (60 mg, daily); (2) anti-platelet: ozagrel (80 mg, *i.v.*, *bid*) or clopidogrel (75 mg, orally, *qd*); (3) dehydrant: mannitol (20 g, *i.v.*, *bid*; Table 1).

Animals

Twelve Cynomolgus monkeys (six female and six male; aged 3 years $\pm$ 2 months) weighing about 3.0 ± 0.5 kg were used in this experiment. Animals were provided by Xishan Zhongke Institute (Suzhou, China) and were housed with controlled temperature (23–25°C) and lighting (8:00–20:00 light, 20:00–8:00 dark) with free access to food and drinking water. All animals were used in accordance with the guidelines of Second Military Medical University for Animal Care.

Cerebellar Infarction Operation in Cynomolgus Monkeys

The Cynomolgus monkeys were anesthetized with pentobarbital sodium (18 mg/kg, *i.p.*). After shaving operation site and disinfection, thoracotomy was performed on the left side. The left vertebral artery and the right brachiocephalic trunk were exposed and ligated for 40 min, followed by complete reperfusion as described previously [14–16]. Then thorax was closed, and the air was removed. Animals were returned to the room with single cage rearing at the previously mentioned condition. Then the animals were administrated LMWH (Pfizer, New York, NY, USA) 147 μ /kg or vehicle (twice a day, *n* = 6 in each group) for 14 days. Other two animals were performed similar sham operation without artery clipping.

Neurological Deficits Score

Neurological deficits were examined using a skeletal muscle coordination function score method [17]. Animals with normal motor function were scored as 0, slightly asynergic movement and abnormal gait as 4, severely asynergic movement as 6, ability to stand, but easy falling down during movement as 10, ability to stand and inability to move as 12, only lying and inability to sit as 16, and no spontaneous motor activity or death as 18.

Morphological Examination and Water Content Measurement

Fourteen days after operation, the Cynomolgus monkeys were anesthetized again. The cerebella and brainstem were removed immediately, and the right cerebella was dried for the measurement of water content [(wet weight–dry weight)/wet weight $\times$ 100%]. The left cerebella and brainstem were immersed in the solution of 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4), and then the tissue was dissected and fixed in this solution for 24 h. The specimens were then washed, dehydrated in a graded ethanol series, and embedded in paraffin. Sections cut transversely at 5 μ m thickness were stained with hematoxylin and eosin (HE) for light microscopic study.

Statistical Analysis

Data are expressed as the mean $\pm$ SD. Data were analyzed by Student's *t*-test for pair-wise comparison. *P* < 0.05 was considered statistically significant.

Results

Anticoagulation Therapy May Be Harmful to Those with Large-Sized Cerebellar Infarction

There are 30 patients (27 men and three women) with large-sized cerebellar infarction (infarct diameter > 3 cm) with or without medulla involvement (Table 1, Figures 1 and 2). Among these 30 subjects, 24 patients (aged 25–78) survived and six (aged from 49–78) died during the following treatment. There is no difference in the infarct volumes confirmed by DW MRI between the death and survival subjects (Table 1). Twenty-eight subjects received the anti-platelet therapy and 27 received dehydrant therapy. All the death subjects received anticoagulation therapy, while nobody in the survival group received such a therapy (Table 1).

The Harmful Effects and Pathological Changes During Anti-Coagulation Therapy in Severe Cerebellar Infarction of Cynomolgus Monkeys

Compared with sham-operated animals, all six cerebellar-infarcted monkeys have obvious neurological deficits (6 ± 0.9 vs. 0), which might be related to the loss of Purkinje cells. Purkinje cells exit in the cerebella and are a prerequisite for normal motor function, and even minor disturbances cause uncoordinated movements and ataxia [18, 19]. Indeed, microscopically, our data showed abnormal morphology in Purkinje cells in cerebellar infarction monkeys. The number and size of the Purkinje cells were reduced, and the nuclei of these cells were also heavily stained and condensed (Figure 3). All these ensure the success of cerebellar infarction in Cynomolgus monkeys.

Two animals in the LMWH group (33%) died on day 10 and day 12, while all animals in the vehicle control group survived (Figure 4A). The animals treated with LMWH had significantly worse overall neurological function (neurological score: 10 ± 2.7

Table 1 Clinical data of 30 patients with cerebellar infarction

No.	Age	Gender	Infarct area (mm)	Medulla involved	Risk factors	Accompanying conditions	Complications	Anticoagulation therapy (LMWH)	Anti-platelet therapy	Dehydrant therapy (Mannitol)	Outcome
1	55	M	34 × 30	Yes	S			Yes		Yes	Survival
2	61	M	32 × 21	Yes	H, S, DM, AF			Yes	Yes	Yes	Survival
3	78	M	69 × 46	Yes	H, S	Bronchitis, Pneumonia		Yes	No	Yes	Death
4	56	M	19 × 34	No	H, S	Myocardial ischemia		Yes	Yes	Yes	Death
5	49	M	MRI unavailable		H		Lung infection	Yes	Yes	Yes	Death
6	56	M	MRI unavailable		H, DM			Yes	Yes	Yes	Death
7	60	M	53 × 32	Yes	H, DM	Coronary stent	Lung infection	Yes	Yes	Yes	Death
8	77	F	41 × 44	Yes	H			Yes	Yes	Yes	Death
9	41	M	41 × 42	No	H, L	Hyperthyroidism	Lung infection	No	Yes	Yes	Survival
10	76	M	49 × 44	Yes	H, AF		Lung infection	No	Yes	No	Survival
11	51	M	64 × 35	Yes	H, DM		Lung infection electrolytes disturbance	No	Yes	Yes	Survival
12	40	F	52 × 44	No			Electrolytes disturbance	No	Yes	Yes	Survival
13	78	F	33 × 38	No	H, AF			No	Yes	No	Survival
14	25	M	40 × 24	No	S, Genetic			No	Yes	No	Survival
15	74	M	33 × 36	No	H, Genetic	Coronary stent		No	Yes	Yes	Survival
16	68	M	68 × 45	No	H, S			No	Yes	Yes	Survival
17	74	M	61 × 53	Yes	S, H	Bronchitis		No	Yes	Yes	Survival
18	76	M	40 × 32	No	H, AF		Lung infection, Hemorrhagic transformation	No	Yes	Yes	Survival
19	41	M	42 × 35	No				No	Yes	Yes	Survival
20	66	M	41 × 35	Yes	H, DM, S	Coronary stent		No	Yes	Yes	Survival
21	61	M	42 × 42	No	S		Lung infection	No	Yes	Yes	Survival
24	74	M	32 × 34	Yes	DM,			No	Yes	Yes	Survival
25	74	M	61 × 61	Yes	S			No	Yes	Yes	Survival
26	45	M	55 × 37	No	Alcoholism	Rheumatic heart disease		No	Yes	Yes	Survival
27	67	M	53 × 35	No	S	Coronary heart disease		No	Yes	Yes	Survival
28	47	M	50 × 40	No	L, S			No	Yes	Yes	Survival
29	72	M	60 × 50	Yes	H	Hepatic abnormality	Lung infection, Tracheotomy		Yes	Yes	Survival
30	60	M	43 × 45	Yes	H	Hepatic abnormality	Lung infection		Yes	Yes	Survival

M, Male; F, female; DM, diabetes mellitus; L, high blood lipid; H, hypertension; S, smoking; AF, atrial fibrillation; LMWH, low molecular weight heparin.

Figure 1 The image photographs of T2 MRI, CT scan, or DW MRI of four patients (Cases 3, 4, 7 and 8) with cerebellar infarction (arrow), who died during the following anti-coagulation therapy.

vs. 6 ± 0.9 in vehicle controls, $P < 0.05$). The water content in the cerebella was also significantly higher in the LMWH group than in the vehicle group ($87.7\% \pm 1.6\%$ vs. $82.8\% \pm 1.0\%$, $P < 0.05$; Figure 4B and C).

The pathological section showed that, not only severer edema but also scattered or diffuse hemorrhage and even subarachnoid hemorrhage occurred in the cerebella as well as brainstem of all the LMWH-treated animals (Figure 4D and E), which might aggravate the cerebellar injury and the brainstem compression.

Discussion

In this retrospective clinical study, the biggest difference in therapy between the death and survival subjects is the presence of anti-coagulation. Six subjects with large cerebellar infarction that received anticoagulation therapy died, while no patient died in the absence of anticoagulation [9]. Similarly, the

mortality rate from the pooled data from six series of consecutive, unselected patients was also significantly lower (7%, 38 of 546) than our data [2]. We definitely show that anticoagulation therapy is harmful, even fatal to patients with large-sized cerebellar infarction.

Hemorrhagic transformation (HT) is a well-known consequence of acute ischemic stroke, but little is known about this complication in cerebellar infarction. Chaves *et al.* found that the causes, imaging findings and consequences of hemorrhagic infarction in the posterior circulation are similar to those in the anterior circulation [20]. Sakamoto *et al.* reported that half of their patients (42/96) showed HT on follow-up T2*-gradient echo imaging, and that both infarct diameter >2.7 cm and volume >4.5 cm³ were independent factors associated with HT [21]. Recently, LMWH therapy has been reported to cause the HT in basilar artery occlusion [22] and lead to severe hemorrhage in preterm neonates [23]. Now, in monkey cerebellar infarction models, hemorrhage and even subarachnoid hemorrhage are present in the LMWH

Figure 2 The image photographs of T2 MRI, CT scan, or DW MRI of part of patients (Case 9–16) with cerebellar infarction (arrow), who survived during the following therapy without anti-coagulation therapy.

Figure 3 Abnormal morphology in Purkinje cells was induced by bilateral cerebellar ischemia operation for 40 min. Section with hematoxylin and eosin (H&E) stain shows: (A) and (B), the normal form of Purkinje cells in the cerebella. (C) and (D), the abnormal form of Purkinje cells in cerebella of cerebellar ischemia animals. Scale bar, 50 μ m.

treatment group, but not in the vehicle group. These data indicate that LMWH may also cause the HT in large-sized cerebellar infarction.

The cerebellum and brainstem are located at the tightly constrained posterior fossa and are bound above by tentorium cerebelli and below by the base of skull. Because of these ana-

Figure 4 Low molecular weight heparin (LMWH) aggravated the cerebellar ischemic injury in Cynomolgus monkeys. **(A)** The survival rate in animals with bilateral cerebellar ischemia operation, which were administrated LMWH or vehicle for 14 days. **(B)** The animals treated with LMWH had significantly worse neurological function. **(C)** The water content was significantly higher in the LMWH group. **(D)** and **(E)** Section with hematoxylin and eosin (HE) stain showed scattered bleeding or splinter hemorrhage (blue arrow), subarachnoid hemorrhage (yellow arrow) and perivascular edema (blue arrow). Liquefaction necrosis (yellow arrow) both in cerebella **(D)** and in brainstem **(E)** of animals treated with LMWH. Seven to 12 is the number of all six monkeys in the LMWH group. Scale bar, 50 μ m. Data are expressed as mean $\pm$ SD. *represent $P < 0.05$, Student's *t*-test.

tomical features, swelling from edema after cerebellar infarction can easily compress the fourth ventricle or the aqueduct of Sylvius, and impede the flow of cerebrospinal fluid, and finally leading to the obstructive hydrocephalus. This natural pathological process of cerebellar infarction itself is very dangerous, and HT, no matter how heavy it is, will certainly make the condition from bad to worse, and consequently compress the brainstem fatally. This might be the main reason to explain the harmful effect of anticoagulation treatment on severe cerebellar infarction.

In our opinion, the critical problem in the management of large-sized cerebellar infarction is to protect or save the medulla oblongata, which is the “center of life”, rather than to treat cerebella itself. Clinicians should familiarize themselves with this important adjoining correlation of medulla oblongata

and the cerebellum. When patients are suspected to suffer from cerebellar infarction, MRI, especially DW MRI, should be performed as early as possible to evaluate its infarct diameter and volume, and its positional relationship with brainstem, and then to choose the most suitable therapy to avoid fatal complications.

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Conflict of Interest

The authors declare no conflict of interest.

References

- Centers for Disease Control and Prevention (CDC). Prevalence of stroke—United States, 2005. *MMWR Morb Mortal Wkly Rep* 2007;**56**:469–474.
- Edlow JA, Newman-Toker DE, Savitz SI. Diagnosis and initial management of cerebellar infarction. *Lancet Neurol* 2008;**7**:951–964.
- Adams HP Jr, del Zoppo G, Alberts MJ, et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: the American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. *Circulation* 2007;**115**:e478–e534.
- Adams HP Jr. Emergent use of anticoagulation for treatment of patients with ischemic stroke. *Stroke* 2002;**33**:856–861.
- Caplan LR. Resolved: heparin may be useful in selected patients with brain ischemia. *Stroke* 2003;**34**:230–231.
- Donnan GA, Davis SM. Heparin in stroke: not for most, but the controversy lingers. *Stroke* 2003;**34**:232–233.
- Moonis M, Fisher M. Considering the role of heparin and low-molecular-weight heparins in acute ischemic stroke. *Stroke* 2002;**33**:1927–1933.
- Sandercock P. Full heparin anticoagulation should not be used in acute ischemic stroke. *Stroke* 2003;**34**:231–232.
- Berge E, Abdelnoor M, Nakstad PH, Sandset PM. Low molecular-weight heparin versus aspirin in patients with acute ischaemic stroke and atrial fibrillation: a double-blind randomised study. Haest study group. Heparin in acute embolic stroke trial. *Lancet* 2000;**355**:1205–1210.
- The International Stroke Trial (IST): A randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. International stroke trial collaborative group. *Lancet* 1997;**349**:1569–1581.
- Low molecular weight heparinoid, org 10172 (danaparoid), and outcome after acute ischemic stroke: a randomized controlled trial. The publications committee for the trial of org 10172 in acute stroke treatment (toast) investigators. *JAMA* 1998;**279**:1265–1272.
- Al-Sadat A, Sunbuli M, Chaturvedi S. Use of intravenous heparin by North American neurologists: do the data matter? *Stroke* 2002;**33**:1574–1577.
- Wang QS, Chen C, Chen XY, et al. Low-molecular-weight heparin versus aspirin for acute ischemic stroke with large artery occlusive disease: subgroup analyses from the fraxiparin in stroke study for the treatment of ischemic stroke (fiss-tris) study. *Stroke* 2012;**43**:346–349.
- Yamashima T, Saido TC, Takita M, et al. Transient brain ischaemia provokes Ca^{2+} , PIP_2 and calpain responses prior to delayed neuronal death in monkeys. *Eur J Neurosci* 1996;**8**:1932–1944.
- Yamashima T, Kohda Y, Tsuchiya K, et al. Inhibition of ischaemic hippocampal neuronal death in primates with cathepsin B inhibitor ca-074: a novel strategy for neuroprotection based on 'calpain-cathepsin hypothesis'. *Eur J Neurosci* 1998;**10**:1723–1733.
- Hossmann KA, Grosse Ophoff B. Recovery of monkey brain after prolonged ischemia. I. Electrophysiology and brain electrolytes. *J Cereb Blood Flow Metab* 1986;**6**:15–21.
- Kito G, Nishimura A, Susumu T, et al. Experimental thromboembolic stroke in cynomolgus monkey. *J Neurosci Methods* 2001;**105**:45–53.
- Girard M, Lariviere R, Parfitt DA, et al. Mitochondrial dysfunction and purkinje cell loss in autosomal recessive spastic ataxia of Charlevoix-Saguenay (arsacs). *Proc Natl Acad Sci U S A* 2012;**109**:1661–1666.
- Sotelo C, Alvarado-Mallart RM. The reconstruction of cerebellar circuits. *Trends Neurosci* 1991;**14**:350–355.
- Chaves CJ, Pessin MS, Caplan LR, et al. Cerebellar hemorrhagic infarction. *Neurology* 1996;**46**:346–349.
- Sakamoto Y, Kimura K, Iguchi Y, Shibasaki K, Aoki J. Hemorrhagic transformation in acute cerebellar infarction. *Cerebrovasc Dis* 2011;**32**:327–333.
- Hsiao MC, Lee JD, Lee M, Weng HH. Massive pontine hemorrhagic transformation associated with an anticoagulant for basilar artery occlusion. *Neurol India* 2006;**54**:431–433.
- van Elteren HA, Te Pas AB, Kollen WJ, Walther FJ, Lopriore E. Severe hemorrhage after low-molecular-weight heparin treatment in a preterm neonate. *Neonatology* 2011;**99**:247–249.