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Full Length Research Paper

Impact of mild hypothermia after ischemia/reperfusion on the cerebral blood perfusion of rats with chronic cerebral hypoperfusion

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This study aimed to investigate the impact of mild hypothermia on cerebral blood perfusion in rats with chronically hypoperfused brain. After ischemia/reperfusion, 12 male healthy Sprague Dawley (SD) rats were randomly divided into normal temperature group (group NT, n=6) and mild hypothermia group (group MH, n=6). The cerebral hypoperfusion model was successfully established by end-to-end anastomosis of the right common carotid artery and the right external jugular vein. After hypoperfusion for 6 weeks, blood perfusion restored, and rectal temperature was maintained at 37°C with a controllable incubation pad in the NT group, while the rectal temperature was maintained at 32°C in the MH group. Rats were re-warmed 3 h after reperfusion. Local cerebral blood perfusion (LCBP) was determined with a laser Doppler flow perfusion imager before reperfusion (T1), immediately after reperfusion (T2) and 48 h after reperfusion (T3). Rats were sacrificed 48 h after reperfusion to observe the ultramicrostructure of brain tissues under transmission electron microscope. In the MH group, LCBP was slightly decreased immediately after reperfusion, and LCBP 48 h after reperfusion restored to the baseline before reperfusion. However, LCBP was significantly decreased immediately and 48 h after reperfusion in the NT group, and the LCBP did not restore to the baseline before reperfusion. In both groups, brain cells at the reperfusion side were swollen, degenerated or even necrotic at different degrees, but the degree of brain damage was slighter in the MH group. The results indicate appropriate early-stage mild hypothermia which may reverse cerebral hypoperfusion and ischemia in rats with chronic cerebral hypoperfusion after reperfusion.

Key words: Chronic cerebral hypoperfusion, mild hypothermia, laser Doppler flow perfusion imager.

INTRODUCTION

Normal perfusion pressure breakthrough (NPPB) of chronic cerebral hypoperfusion after ischemia/reperfusion is an important factor resulting in cerebral hemorrhage and edema, which seriously restrict the prognosis of patients (Friedlander, 2007). Postoperative NPPB may be caused by the sudden increase of blood perfusion in brain tissues around the intracranial arteriovenous malformations (Li et al., 2009).

Recently, pathophysiological, cytophysiological and clinical research showed that, mild hypothermia played protective roles in cerebral ischemia and hypoxia (Polderman, 2008). In this study, a rat model of chronic cerebral hypoperfusion model after ischemia/reperfusion were established, and laser Doppler flow perfusion imaging was used to investigate the effects of mild hypothermia on cerebral blood perfusion in chronic cerebral hypoperfusion rats after ischemia/reperfusion.

MATERIALS AND METHODS

Animals and grouping

Fifteen healthy male Sprague-Dawley rats weighting from 210 to

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Table 1. Changes of heart rate, mean arterial pressure and breathe rate before and after hypothermia ($\bar{x} \pm s$).

Group		Before hypothermia (T:37℃)	After hypothermia (T:32℃)	*P*
NT group	HR (bpm)	380.84±40.56	384.71±22.27	0.836
(n=6)	MAP (mmHg)	103.34±11.53	100.43±4.78	0.142
	RR (bpm)	76.42±1.22	77.35±2.38	0.125
MH group	HR (bpm)	376.59±35.59	398.09±41.79	0.061
(n=6)	MAP (mmHg)	96.04±2.67	91.83±1.79	0.012*
	RR (bpm)	71.06±1.42	72.24±2.19	0.367

Compared with the NT group, * $p<0.05$.

230 g were purchased from Animal Laboratory Center of Sun Yat-sen University and randomly divided into two groups: a normal temperature group (group NT, n = 6) and a mild hypothermia group (group MH, n = 6). Another 3 rats served as control. Before and after modeling, all laboratory animals were bred in the SPF laboratory of Animal Laboratory Center of Sun Yat-sen University. The study was approved by the institutional review boards of the Sun Yat-sen University.

Modeling

According to Yassari's method (Yassari et al., 2004), we modified the chronic cerebral hypoperfusion rat model after ischemia/reperfusion. Rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (1 ml/kg), and then fixed in the supine position on an operation table. Skin at anterior middle cervical triangle was slit to expose left and right external jugular veins (EJV) and the common carotid arteries (CCA), and the distal end of the right CCA and the proximal end of the right EJV was anastomosed with 11.0 nylon vascular suture under microscope. Meanwhile, right and left EJV were occluded. During rate modeling, peak blood flow rate of contralateral common carotid artery was continuously determined. After recanalization of the anastomosed blood vessels, the peak blood flow rate decreased by more than 25% indicating obvious blood loss effects and thus the cerebral hypoperfusion rat model was successfully established.

After hypoperfusion for 6 weeks, the anastomosed blood vessels were disconnected to restore blood reperfusion. Rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (1 ml/kg), and the rectal temperature was continuously monitored. Rectal temperature was maintained at 37℃ with a controllable incubation pad in the NT group, while rectal temperature at 32℃ was induced by physical cooling with alcohol and maintained with a controllable incubation pad in the MH group. Rats were naturally re-warmed 3 h after reperfusion (Kawahara et al., 2003; Lavinio et al., 2007). Laboratory temperature was maintained at 24 to 25℃ and humidity at 60%.

Sampling

Rats were fixed on a stereotaxic apparatus to receive craniotomy, and a craniotomy window with a size of 5 × 6 mm was made between coronal suture and lambdoid suture. Local cerebral blood perfusion (LCBP) of exposed cerebral cortex area was continuously determined with a laser Doppler flow perfusion imager (LDPI, PeriScan PIM 3, Perimed Company, Sweden) by low-frequency laser beam before reperfusion (T1), immediately after reperfusion (T2) and 48 h after reperfusion (T3). The parameters of LDPI included laser wavelength at 670 nm, step length at 3 mm, NR scanning model, scanning head height at 15 to 30 cm and scanning area at 4 × 4 cm. Mean perfusion volume (PU) of LCBP curve was calculated. Test data and images were stored on computer hard disk and analyzed with LDPI win 3.1 software, and the analytical results were imported into LDPI win 3.1 for comparative analysis (Kimme et al., 2002).

48 h after reperfusion, LDPI was determined, and then rats were perfused and fixed with 2% glutaraldehyde and 4% paraformaldehyde solution. About 2 × 2 × 2 mm brain cortical tissue near median line in the ischemia side was harvested, fixed with 2.5% glutaraldehyde, dehydrated with acetone and embedded with ethoxyline resin. Ultrathin sectioning was performed, and sections were repeatedly stained with uranyl acetate and lead citrate and observed under transmission electron microscope. 12 rats served as test and 3 rats served as control.

Statistical analysis

Statistical analysis was performed with SPSS version 11.0 statistical software. Measurement data were expressed as mean ± standard deviation (X ± s). Intragroup comparison was done with ANOVA, and group comparison was done with t test, and $p<0.05$ was considered statistically significant.

RESULTS

The mean arterial pressure in the MH group was significantly decreased from 96.04 ± 2.67 to 91.83 ± 1.79 mmHg when the rectal temperature changed from 37℃, at 32℃ ($p<0.05$). However, there were no significant differences in heart rate or breath rate between both groups (Table 1). In the NT group, after the artery was occluded, there was no significant difference in cerebral blood flow volume compared with that before occlusion, but the cerebral blood flow volume was significantly decreased 48 h after occlusion ($p<0.001$). In the MH group, after the artery was occluded, the cerebral blood flow volume was significantly decreased compared with that before occlusion ($p<0.05$), but the cerebral blood flow volume 48 h after occlusion restored to the level before occlusion ($p>0.05$), indicating that after occlusion, the cerebral blood flow volume in the MH group was first decreased but was eventually gradually restored to pre-occlusion level.

In the NT group, there was no significant change on the

Table 2. Changes of rat cerebral blood flow volume before and after reperfusion ($\bar{x} \pm S$) PU.

Group		T1	T2	T3
NT group (*n*=6)	LCBP (PU)	278.23±38.07	265.69±36.45	194.74±5.32 #
MH group (*n*=6)	LCBP (PU)	221.49±18.56*	202.37±7.98*	212.72±7.89

Compared with the NT group, * p<0.05; compared with the subgroups T1 or T2, # p<0.001.

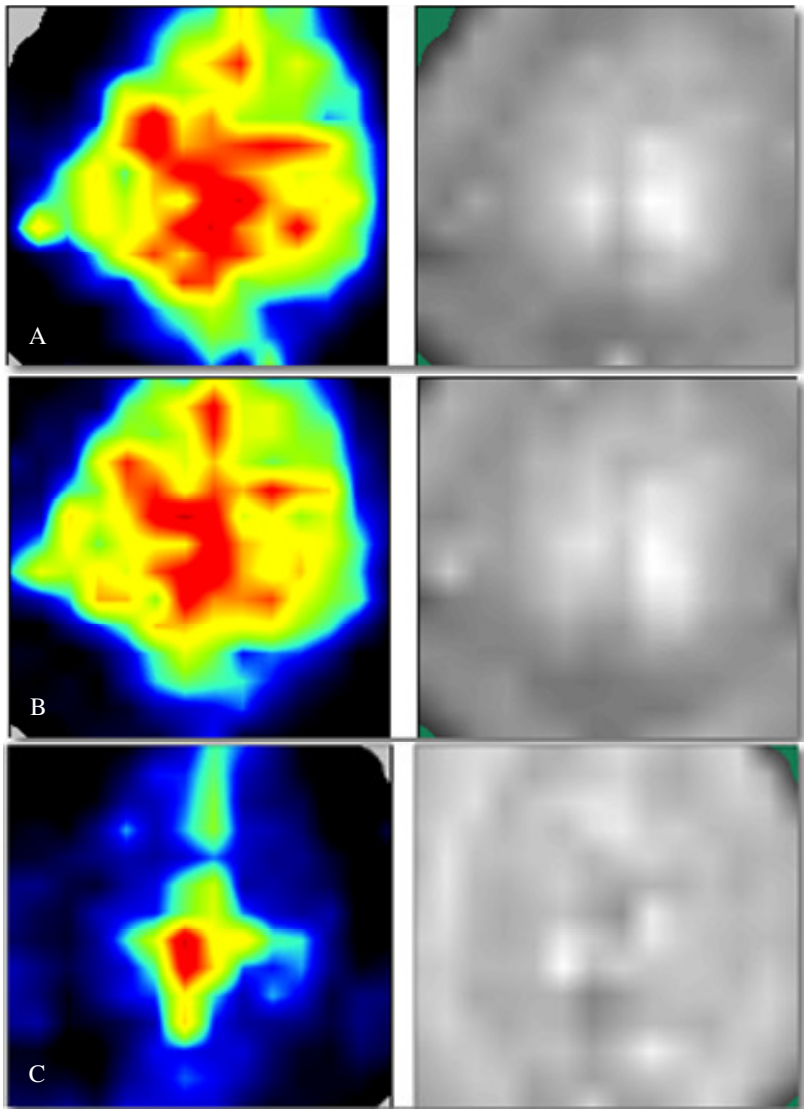

Figure 1. Local cerebral blood perfusion (LCBP) in the NT group. **A**: Rat cerebral blood flow volume before occlusion; **B**: LCBP of exposed cerebral cortex area immediately after occlusion; **C**: LCBP of exposed cerebral cortex area PU 48 h after occlusion. Left: perfusion volume image of the determined area, blue indicates low perfusion volume, red indicates high perfusion volume; Right: the determined area.

cerebral blood flow volume immediately after occlusion; however, this was significantly decreased 48 h after occlusion (Table 2, Figures 1 and 2). In healthy rats, the structure of brain cells and capillary endothelial cells was intact, and there was no obvious change in intervascular connections, and foot processes of glial cells were not hydropic (Figure 3A). In the NT group, after reperfusion for 48 h, brain cells were significantly swollen in varying

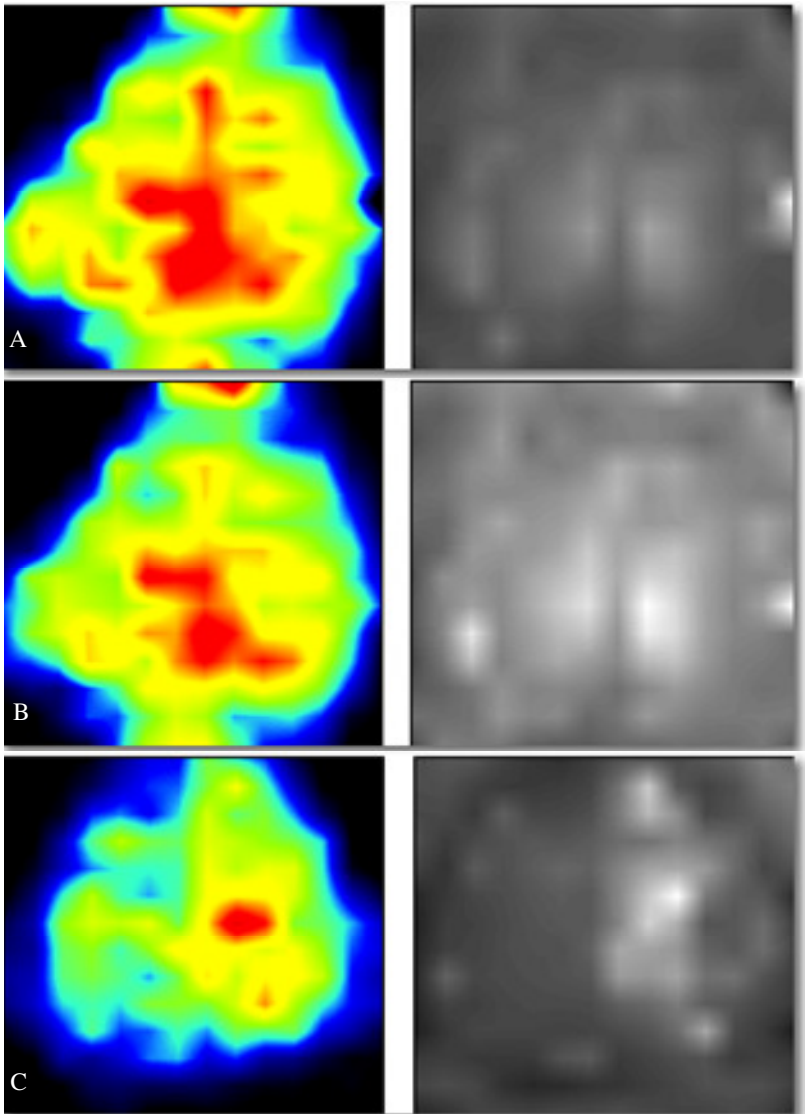

Figure 2. Local cerebral blood perfusion (LCBP) in the MH group. A: LCBP of exposed cerebral cortex area before occlusion; B: LCBP of exposed cerebral cortex area immediately after occlusion; C: LCBP of exposed cerebral cortex area 48 h after occlusion. Left: perfusion volume image of the determined area, blue indicates low perfusion volume, red indicates high perfusion volume; Right: the determined area.

degrees, and most tight junction between capillary endothelial cells was open, and nerve cells and glial cells were degenerated, swollen, or even necrotic (Figure 3B). In the MH group, the damage of brain cells and blood-brain barrier was slight, and the opening of close connections was not obvious, and perivascular tissues were mildly hydropic (Figure 3C).

DISCUSSION

Chronic hypoperfusion usually results in diffuse brain edema and (or) intracranial hemorrhage with high fatality and disability rate due to the restoration of normal perfusion pressure and change of ischemic state. Reperfusion following cerebral hypoperfusion is the starting point of the cascade reaction of brain damage, and it is also the best treatment period. Hence, we believe that mild hypothermia given before reperfusion as in this study might be useful in preventing further damage. The chronic cerebral ischemia animal model established in this study was similar with the cerebral blood flow state of human brain arteriovenous malfo-mation (BAVM). This was done to simulate the blood flow change of surrounding brain tissues after resection of BAVM, to uncover the degree of cerebral ischemia and

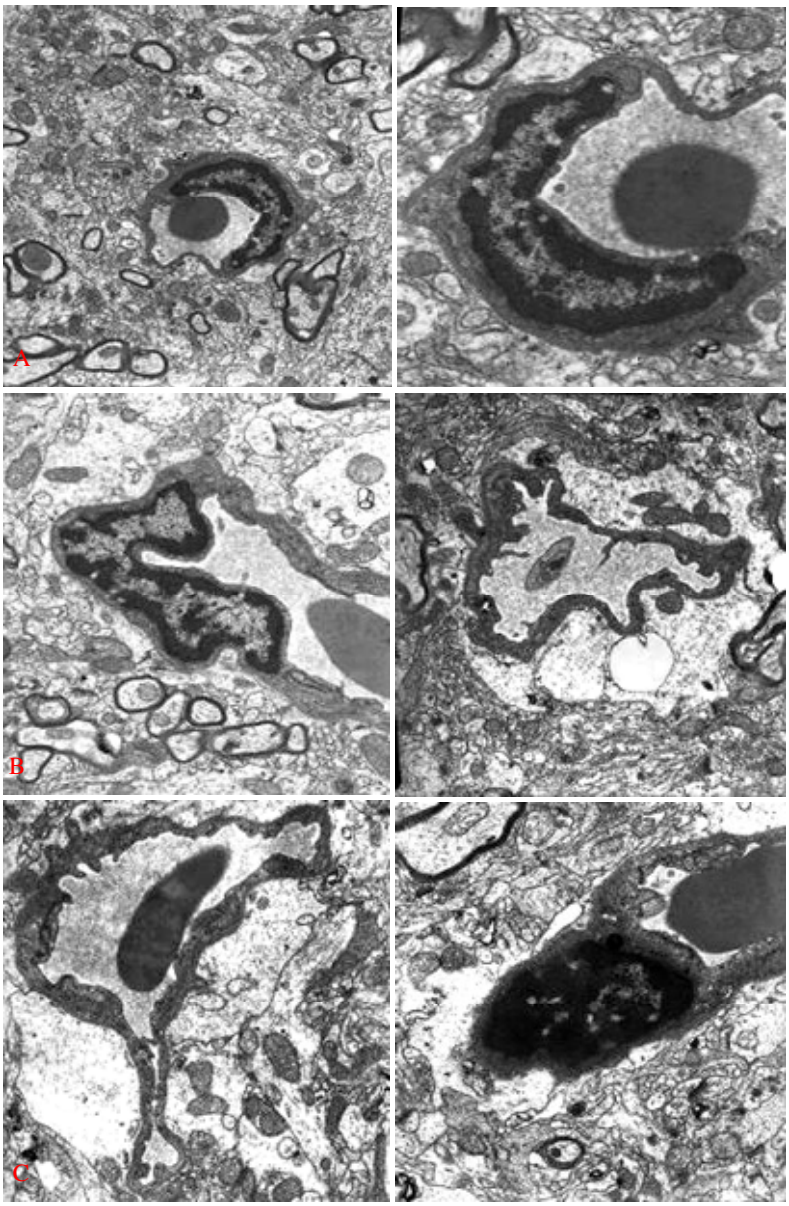

Figure 3. Electron microscopy. A: The findings in the control group. Blood-brain barrier was normal, and the structure of capillary endothelial cells was intact, and foot processes of glial cells were not hydropic. Left: (×6000), right: (×15000); B: The findings in the NT 48 h subgroup. There were obvious vacuoles around blood vessels, and blood-brain barrier was damaged, and basement membrane was incomplete, and extravascular tissues were obviously swollen. Left: (×6000); Right: (×15000); C: The findings in the MH 48 h subgroup. Perivascular tissues were mildly hydropic, and some brain cells were necrotic, and cytoplasm and nuclei of some glial cells was apoptosis, and cell organelle was expanded due to enhanced cellular cell organelle, and mitochondriae were mildly swollen. Left: (×6000), right: (×15000).

perfusion, and to predict the possibility of the complications such as postoperative intracranial hemorrhage and edema. Laser Doppler perfusion imaging (LDPI) is considered as a real-time, continuous, non-invasive and sensitive monitoring technology on microcirculation blood flow, which is suitable for neurosurgical intraoperative monitoring of the local blood flow of cerebral cortex, and especially for comparison of the relative changes of blood perfusion volume.

In this study, immediately after reperfusion, local blood

perfusion of cerebral cortex was only slightly decreased by 5%. With the length of blood perfusion time, local blood perfusion was decreased to 70% of baseline level before perfusion and did not restore to the baseline level. These results indicate that, in the early stage, the local blood perfusion of chronically hypoperfused cerebrum of rats might depend on the increased venous pressure during reperfusion period. Also, it appears that the perfusion pressure normalization during reperfusion period would result in appropriate reactive high blood volume. When the restored perfusion pressure overcome the tolerance of microcirculation, increased blood vessel permeability of microcirculation resulted in vasogenic edema and persistent cerebral hypoperfusion, and eventually leading to dysfunction of the blood flow autoregulation during reperfusion period.

Reducing the reperfusion blood flow is crucial to prevent postoperative edema of BAVM, and in clinical practice reperfusion blood flow was reduced by surgical deligation and embolization of feeding artery and controlled hypotension, but these measurements did not prevent the occurrence of NBBP. At present, mild hypothermia is a more feasible and promising treatment method on hypoxic-ischemic brain injury (Polderman, 2008).

Animal studies have shown that, during and after reperfusion period following cerebral ischemia, mild hypothermia may have significant neuroprotective effects, and may prolong the therapeutic window. No significant adverse reactions were found in small-scale clinical studies, showing a degree of neuroprotective effect (Schaller and Graf, 2004; Zausinger et al., 2003). With the application of therapeutic mild hypothermia on cerebral trauma, cerebral infarction and cerebral aneurysm, the beneficial effects of hypothermia on the cerebral blood flow and metabolic changes may gradually attract more and more people's attention (Strazevska et al., 2008; Kimberger and Kurz, 2008).

Cerebral protective effects of mild hypothermia might be due to effects on multiple links of cerebral ischemia and hypoxic injury such as to regulate cerebral blood flow, decrease the needs of brain cellular metabolism, reduce the release of excitatory amino acids, and postpone and (or) reduce the apoptosis of nerve cells. Studies on how cerebral blood flow changes after reperfusion in mild hypothermia state are crucial for the clinical application of mild hypothermia treatments on chronic cerebral ischemic reperfusion injury. The changes of MAP during temperature decrease in this study indicate that, the changes of cerebral blood flow (CBF) might be related to the MAP during temperature decrease, and total body hypothermia might result in the change of blood circulation. A rectal temperature of 32°C in this study not only resulted in no further decrease of CBP of reperfusion rats but also improved local cerebral blood flow after reperfusion.

Therefore, we believe that mild hypothermia at 32°C is safe in clinical practice. It was observed in this study that there was no significant change in cerebral blood perfusion immediately after reperfusion in the NT group, and cerebral blood flow perfusion decreased 48 h after reperfusion, indicating that cerebral blood flow perfusion increasingly decreased with the length of reperfusion time. Moreover, ultrastructural observation of brain tissues revealed that blood brain barrier was severely damaged, and endothelial cells were hydropic, and mitochondria of nerve cells and glial cells were swollen, indicating reperfusion following chronic cerebral ischemia had induced some degree of ischemic and hypoxia changes. In the MH group, although there was mild decrease in cerebral blood perfusion immediately after reperfusion, however, this was almost restored to near normal values or baseline values 48 h after reperfusion, indicating that mild hypothermia could shorten the time of perfusion recovery. Moreover, ultrastructure observation of brain tissues revealed that there was no further damage on blood brain barrier, and the brain cells were only slightly hydropic. Therefore, it was necessary to continuously monitor the changes of cerebral blood flow in the clinical practice of mild hypothermia and set an appropriate target temperature of mild hypothermia.

Conclusion

In conclusion, cerebral blood perfusion of chronic cerebral ischemia model rats after reperfusion presented persistent low perfusion. Mild hypothermia was conducive to improve the cerebral blood perfusion of chronic cerebral ischemia rats after reperfusion, and shorten the time of perfusion recovery, and alleviate brain tissue damage. The study simulated the clinical course of cerebral arteriovenous malformation resection. The lesion perfusion pressure will be gradually increased with the fractional flow in lesions. After removing the deformities, blood flow increased dramatically and the vertical stress of the vascular wall increases, leading to overload and reperfusion injury. Hypothermia to reduce the reperfusion of cerebral blood flow after ischemia and reperfusion can prevent excessive perfusion, reduce the damage of high perfusion pressure on the blood-brain barrier and reduce the incidence of complications such as brain edema and cerebral bleeding.

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