Enhanced Neuroprotection by Local Intra-Arterial Infusion of Human Albumin Solution and Local Hypothermia

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Background and Purpose—We investigated the potential benefit of using a local infusion of low-dose and cold human albumin in ischemic rats as compared with systemic delivery.

Methods—Stroke was induced in rats, and at 2 hours treatment groups received 0°C saline or low-dose albumin at 0°C or 37°C infused into the ischemic area.

Results—The local low-dose cold albumin infusion, which achieved the hypothermic temperature (P<0.001), produced the greatest reduction in infarct volume and the best recovery of neurological function.

Conclusions—The local low-dose cold albumin infusion into the ischemic area offered a combination of regional brain hypothermia and albumin administration, which enhanced neuroprotection and would be beneficial in the clinical setting. (Stroke. 2012;44:00-00.)

Key Words: brain infarction ■ hypothermia ■ neuroprotective agents ■ stroke ■ therapeutics

Recently, both moderate and high-dose systemic human Alb therapy have been shown to markedly improve neurological function in clinical stroke studies; however, they were found to cause severe dose-related adverse effects. As such, it was important to determine whether a low-dose of human Alb solution, directly delivered into the ischemic territory, could produce a level of neuroprotection similar to that provided by systemic administration.

We previously demonstrated that robust neuroprotection was provided by inducing regional brain hypothermia, via a cold saline infusion, in the cerebral ischemia area. In addition, similar studies have been performed in humans. In the present study, we examined whether a locally infused cold Alb solution could enhance neuroprotection.

Materials and Methods

Animal Preparation
Male Sprague-Dawley rats (300–340 grams, Vital River Laboratory Animal Technology Co Ltd, China) were studied. All procedures were conducted according to institutional guidelines and were in compliance with regulations formulated by the Animal Care and Use Committee, Capital Medical University of Beijing, China. Body temperature was measured continuously through the rectum, and a heating lamp and pad were used to maintain body temperature at 37.0°C to 37.5°C. Brain temperature and local cerebral blood flow were monitored continuously as described previously.

Experimental Protocol
Two-hour middle cerebral artery occlusion and reperfusion were induced by a modified microcatheter model. Before reperfusion, 0.9% cold (0°C) saline or human Alb (0°C or 37°C; Tian Tan Biotherapeutic Corp, 20% solution) was infused. Animals were randomly divided into 6 groups: (1) non-treatment group (n=8); 2-hour middle cerebral artery occlusion followed by 48 hours of reperfusion; (2) local cold (0°C) saline infusion group (0.9% saline, n=12); (3) local low-dose cold (0°C) human Alb infusion group (0.5 g/kg, n=12); (4) local low-dose normothermic (37°C) human Alb infusion group (0.5 g/kg, n=12); (5) systemic low-dose normothermic (37°C) human Alb infusion group (0.5 g/kg, n=8) applied through the femoral artery; (6) systemic high-dose normothermic (37°C) human Alb infusion group (1.5 g/kg, n=12). All treatments, examinations, and evaluations were performed by investigators blinded to the groups.

Neurological and Behavioral Examination
After ischemia-reperfusion, neurological deficits were scored. Animals were also examined with motor testing paradigms (foot-fault placing, parallel-bar crossing, and ladder and rope climbing).

Cerebral Infarct Volume
Brain slices were treated with 2,3,5-triphenyltetrazolium chloride (TTC, Sigma, St. Louis, MO), and an indirect method for calculating infarct volume was used.

Statistical Analysis
The values are expressed as means±SEM. Differences between 2 groups and differences between multiple groups were evaluated using a Student t test or 1-way ANOVA, respectively, with a significance level at P<0.05. Post hoc comparison between groups was further performed using the least significant difference method.

Results

Regional Brain Hypothermia
In <3 minutes, the local 0°C Alb or saline solution infusion significantly (P<0.01) reduced the temperature of the
cerebral cortex region supplied by the middle cerebral artery from 37.2±0.2°C to 30.5±0.4°C, and in the striatum from 37.8±0.1°C to 30.8±0.4°C, and remained reduced for up to 45 minutes. Body temperatures remained close to baseline. The brain and body temperatures of animals in other groups remained normothermic during ischemia and reperfusion.

Cerebral Lesion Volume
Infarction volume in the nontreatment group was 61.6±10.8%. Although the measured lesion volume in the systemic low-dose normothermic Alb infusion group (52.5±5.6%) was reduced when compared with the nontreatment group, the reduction did not reach a significant level. Compared with the nontreatment group, the lesion volume was 35.4±6.6% (−43%) in animals treated with a local 37°C Alb (0.5 g/kg) infusion, 41.7±6.4 (−32%) with a local 0°C saline infusion, 38.8±5.8 (−37%) with a systemic 37°C Alb (1.5 g/kg) infusion, and 20.3±8.7 (−67%) with a local 0°C Alb (0.5 g/kg) infusion. The local cold Alb infusion resulted in the greatest (P<0.01) reduction in lesion volume (Figure 1).

Functional Outcome
Animals in all groups exhibited severe neurological deficits at 30 minutes after the 2-hour middle cerebral artery occlusion, with no significant differences between groups (Figure 2). After 24 and 48 hours of reperfusion, the systemic low-dose normothermic Alb infusion group failed to demonstrate improved recovery of neurological and motor function (Figures 2 and 3). However, rats in the other 4 groups showed (Figures 2 and 3, P<0.01) improvements in neurological and motor function. Furthermore, the rats that received local cold Alb (0.5 g/kg) infusion therapy had the best recovery (P<0.05).

Discussion
In this study, we demonstrated a synergistic effect of regional brain hypothermia and low-dose Alb infusion in acute ischemic stroke. In several previous clinical trials of preclinically promising neuroprotectants, the substances failed to earn clinical approval because of the pronounced side effects of therapeutic doses.8,9 A recent study suggests that elective intra-arterial drug injection might result in high local drug

Figure 1. The local low-dose cold Alb infusion showed a significant reduction in brain lesion volume as compared with the non-treatment group (**P<0.001) and a further reduction as compared with the local low-dose, systemic high-dose normothermic Alb, and local cold saline groups (*P<0.05). The local low-dose and systemic high-dose normothermic Alb and local cold saline groups also showed a reduction in brain lesion compared with the nontreatment group (*P<0.01). Values are means±SEM.

Figure 2. Neurological scores were comparable during early reperfusion (30 minutes). At 24 and 48 hours after reperfusion, the local low-dose cold Alb infusion group showed significant neurological improvement (P<0.01) compared with the nontreatment group, and a further improvement (*P<0.05) as compared with the local cold saline and the local low-dose and systemic high-dose normothermic Alb groups, which significantly (*P<0.01) reduced neurological deficits at 24 and 48 hours after reperfusion. Values are mean±SEM.
concentrations and reduced systemic adverse effects compared with systemic administration. In the present study, because the Alb solution was directly infused into the ischemic cerebral area, a low dose still conferred neuroprotection as significant as that achieved by systemic administration of high-dose Alb. Clinically, a local low-dose Alb infusion would reduce the risk of adverse dose-related systemic effects seen more frequently with high-dose systemic Alb infusions. In the present study, regional brain hypothermia was induced by local intra-arterial infusion with a small amount of cold Alb solution. This method produced hypothermia much faster than systemically induced whole body hypothermia, so as not to miss the therapeutic window. The present study showed acute neuroprotection, however long-term neuroprotection remains to be investigated. Because a great variety of endovascular tools exist for delivering medications to intracranial vessels, this protocol, combined with intra-arterial thrombolysis or mechanical embolectomy, could be functionally applied in the clinical setting.

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Disclosures
None.

References
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