Protective effect of albumin on VEGF and brain edema in acute ischemia in rats

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ABSTRACT

Vascular endothelial growth factor (VEGF) is known to be an important stroke-related pathogenic factor for the formation of brain edema. We examined the therapeutic effect of human serum albumin on VEGF expression in acute ischemic stroke. Adult male Sprague–Dawley (SD) rats were subjected to Middle Cerebral Artery Occlusion (MCAO), the suture was withdrawn 2 h later, and 25% albumin (1.25 g/kg) or saline (5 ml/kg) was administered intravenously after reperfusion. The model was evaluated by 2,3,5-triphenyl-tetrazolium chloride (TTC) staining, neurological deficits and brain water content. Serum albumin level was determined. VEGF expression was studied by enzyme linked immunosorbent assay (ELISA), quantitative real-time PCR and immunohistochemistry. We demonstrated that albumin administration maintained the serum albumin at a higher level than the sham group at 6 h, 1 d, 2 d and 3 d after MCAO, and significantly improved the neurological deficits and decreased the brain water content. In addition, the strong up-regulation of VEGF expression at 6 h and 1 d after MCAO can be attenuated by albumin administration. However, albumin administration had no significant depressing effect on VEGF expression at 2 d, 3 d and 5 d after MCAO in the cortex and hippocampus. Strong up-regulation of VEGF immunoreactivity was noted in the saline group in the blood–brain barrier (BBB), and in neurons surrounding the peri-infarct area and periventricular area at 24 h after MCAO. The expression of VEGF in the albumin group was much weaker. Furthermore, there were high correlations between the brain water content with the serum albumin level, with serum VEGF protein level, and with brain VEGF mRNA expression at 24 h after MCAO. In conclusion, maintaining the serum albumin at a higher level than the sham group at 6 h, 1 d, 2 d, 3 d, or 5 d after MCAO, may partially contribute to the protective effects of albumin on reduction of brain edema in the early stage of ischemia.

Keywords: Middle Cerebral Artery Occlusion (MCAO), Vascular endothelial growth factor (VEGF), Albumin, Brain edema

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lamps during and after the operation. Arterial blood gases of pH, PaO₂, PaCO₂, and blood pressure were monitored. Rats were subjected to 2 h of focal ischemia induced by left MCAO with a round tip poly-L-lysine-coated monofilament insert. After 2 h of occlusion, the suture was withdrawn, and 25% human serum albumin (1.25 g/kg) or equal volume of 0.9% saline vehicle (5 ml/kg) was administered intravenously at a constant rate over 3 min [1,19]. Sham rats underwent all procedures except for MCAO.

The model was evaluated by the 2,3,5-triphenyl-tetrazolium chloride (TTC) staining and neurological deficits. Neurological examinations were graded as follows: a score of 0 suggests no neurological deficit (healthy); 1 suggests mild neurological deficit (failure to extend right forepaw fully); 2 suggests moderate neurological deficit (circling to the right), 3 suggests severe neurological deficit (falling to the right), and 4 suggests very severe neurological deficit (unable to walk spontaneously with depressed level of consciousness) [22]. Tests were performed by an observer blinded to the treatment group.

Brain water content was measured by the wet and dry weight method [26]. Rats were decapitated under deep anesthesia in sham, albumin and saline group at 24 h after MCAO (N = 5 for each group), following separation of the cerebellum and the olfactory bulb, the cerebrum was divided into hemispheres. The wet weight of the ipsilateral hemisphere was weighed immediately, and then dried at 110°C for 24 h to get the dry weight. The brain water content was calculated as: brain water content (%) = [(wet weight – dry weight)/wet weight] × 100.

Serum samples of each group of rats were extracted. The serum albumin level (g/L) was measured by bromocresol green assay by using spectrometer analyzer (UV1012, America). VEGF protein level in the blood serum was measured by commercially available ELISA kit specific for rat VEGF (Boster Biological Technology Ltd.) according to the manufacturer’s instructions.

Total RNA was extracted and cDNA was reverse transcribed from the ipsilateral brain of hippocampus or cortex. Quantitative real-time PCR was performed (LightCycler 1.5, Roche Diagnostics) by using DNA Master SYBR Green 1 (TaKaRa, Japan). The VEGF primer sequence is 5′-gtttactgctgacccac-3′, 5′-agaagtccgccagac-3′, Tm 55.5°C, 193 bp; GAPDH primer sequence is 5′-tatcgagccttgattac-3′, 5′-cgttcaagttgccgtc-3′, Tm 53.9°C, 140 bp. The amplification program is followed by melting curve analysis. A negative control was included in each run to evaluate the specificity of primers and possible contamination. The relative quantification of VEGF gene was calculated using the following formula: 2−ΔΔCT method [18], ΔΔCT = (CT,VEGF − CT,GAPDH)T ime x − (CT,VEGF − CT,GAPDH)T ime 0 (x = 6 h, 1 d, 2 d, 3 d, 5 d, 0 = sham).

For VEGF immunohistochemistry, sequential slides were incubated with the primary antibody, rabbit polyclonal anti-rat VEGF (1:100), and the biotinylated goat anti-rabbit immunoglobulin sec-
ory, PaO₂, PaCO₂, and blood pressure between groups before and after the operation (p > 0.05).

Albumin administration group showed significantly improved neurological deficit scores compared to saline group at 24 h after MCAO (N = 6 for each group, p = 0.041, non-parametric analysis with Mann–Whitney's test. Median value: 2 for albumin group, 3 for saline group). Accordingly, TTC staining of the albumin group showed a smaller infarct size compared to the saline group as shown in Fig. 1A. There was a significant decrease in brain water content in the albumin group compared to the saline group at 24 h after MCAO (p < 0.01, Fig. 1B). Furthermore, from inspection of the brains, obvious edema was observed in the whole brain in the saline group, especially in the ischemic hemisphere. The tissue of the ischemic hemisphere was degraded and became very soft. In contrast, the brain swelling was attenuated in the albumin group, and the cortical tissue of the ischemic hemisphere remained intact and elastic.

The albumin group showed relatively higher serum albumin levels compared to saline group at 6 h, 1 d, 2 d, 3 d after MCAO (p < 0.05), and there were significant serum albumin level changes in the albumin group at 6 h, 1 d, 2 d, 3 d compared to the sham group (p < 0.05), which suggest that albumin administration maintained the serum albumin level at a higher level than the sham group (Fig. 1D).

VEGF mRNA expression assessed by quantitative real-time PCR showed interesting results. In the cortex, albumin administration significantly depressed the strong up-regulation of VEGF mRNA expression at 6 h and 1 d after MCAO compared to the saline group (p < 0.05). However, although VEGF expression was still up-regulated in both saline and albumin group at 2 d and 3 d after MCAO, there were no significant depressing effect on VEGF expression between the albumin and saline group at 2 d and 3 d after MCAO in the cortex (p > 0.05). In the hippocampus, VEGF mRNA expression was significantly up-regulated in both saline and albumin groups at 6 h and 1 d after MCAO compared to the sham group (p < 0.05). While VEGF expression in the albumin group at 6 h and 1 d after MCAO were significantly lower than in the saline group in the hippocampus (p < 0.05). In addition, there was no significant difference between the albumin and saline group at 2 d, 3 d and 5 d after MCAO (p > 0.05). These data suggest that, the strong up-regulation of VEGF mRNA expression at 6 h and 1 d after MCAO can be attenuated by albumin administration. However, albumin administration has no significant depressing effect on VEGF expression in the cortex and hippocampus at 2 d, 3 d and 5 d after MCAO.

Serum VEGF level was measured by ELISA. Compared to the saline group, the serum VEGF level of albumin group was significantly depressed at 1 d after MCAO measured by ELISA (p < 0.05, Fig. 1C), which suggests that albumin administration may attenuate the strong up-regulation of serum VEGF level at 1 d after MCAO.

Spatial distribution features of VEGF immunoreactivity by immunohistochemistry demonstrated that, there was no obvious alteration in VEGF immunoreactivity in the sham group (Fig. 2A). VEGF immunoreactivity could be observed in both the albumin and saline groups at 1 d after MCAO. Strong up-regulation of VEGF immunoreactivity was noted in the saline group at 1 d after MCAO (Fig. 2B, E–G), especially in the choroid plexus (Fig. 2E) and microvessels (Fig. 2F and G). Following albumin treatment, the immunoreactivity for VEGF was markedly reduced (Fig. 2C) when compared to the saline group (Fig. 2B). In addition to the strong positive VEGF expression in the blood–brain barrier (BBB), such as the choroid plexus, meninges and microvessels, VEGF immunoreactivity was also observed in the neurons (Fig. 2H) surrounding the peri-infarcted area and periventricular area (Fig. 2D) in both saline and albumin group.

There were high correlations between the brain water content with the serum albumin level (r = −0.865, p < 0.01), with serum VEGF protein level (r = 0.858, p < 0.01), and with brain VEGF mRNA expression in hippocampus (r = 0.835, p < 0.01) and in cortex (r = 0.902, p < 0.01) at 1 d after MCAO, which suggests that a decreased serum albumin level, strongly up-regulated serum VEGF protein level or brain VEGF mRNA expression may contribute to greater brain swelling at 1 d after MCAO.

In agreement with previous studies [2], we confirmed the beneficial effect of moderate-dose albumin therapy (1.25 g/kg) administered immediately after reperfusion, markedly reduces brain swelling. The fundamental explanation is that albumin possesses the capacity to draw interstitial fluid into the intravascular space to reduce the undesirable edema [11]. Brain edema may thereby be prevented or significantly reduced.

In addition, we further extended these studies to show that albumin administration maintained the serum albumin at a higher level than the sham group at 6 h, 1 d, 2 d and 3 d after MCAO. Serum...
albumin level is a predictor of the prognosis of ischemic stroke [3]. Human serum albumin (HSA) is the most abundant plasma protein and an important circulating carrier. The multiple beneficial effects of higher serum albumin level include binding to free fatty acids, metabolites and drugs, providing energy to neurons for metabolism and repairing injured neurons, supporting endothelial cells’ function and exerting antioxidant effects [2,23,30]. We suggest that albumin administration may exert its multiple protective properties by maintaining relatively higher serum albumin levels to improve the neurological outcome.

Moreover, the current study highlighted the attenuation effect of albumin administration on VEGF in the serum; in brain mRNA and protein levels during the early stage (1 d) after MCAO. High correlations were indicated between brain water content with VEGF, and with serum albumin level at 1 d after MCAO. Furthermore, strong up-regulation of VEGF immunoreactivity was observed in the saline group, in neurons surrounding the peri-infarct area, and in the endothelial cells of the BBB. While the immunoreactivity of VEGF was much weaker in the albumin group at 1 d after MCAO, which suggests that albumin administration may exert its protection on the reduction of VEGF expression in the above mentioned key places in acute ischemia. It has been reported that albumin exerts multiple actions on vascular endothelium, helping to maintain normal microvascular permeability [7,10]. VEGF upon binding to its receptors on endothelial cells disrupts the organization of interendothelial junctions [11] and the interstitial extracellular matrix (ECM) complexes, thereby opening the junctional barrier. Albumin can associate with surface glycoproteins in endothelial cells and increase the resistance to water and solute flows through hydraulic pathways across the capillary wall.

VEGF has strong vascular permeability. It might induce vaso- genic brain edema in the acute stage of ischemia [9]. Experimental evidence indicates that early postischemic (1 h) administration of rhVEGF165 to ischemic rats significantly increased BBB leakage and ischemic lesions [29]. Antagonism of VEGF has shown to reduce ischemia/reperfusion-related brain edema and injury, suggesting the role for the enhanced microvascular permeability effects of VEGF in the formation of cerebral edema in the acute stage of ischemia [5,27]. We suggest that the reduction of VEGF expression after albumin administration may partially mimic the VEGF antagonist, through decreased vascular permeability and subsequent decrease in brain water content.

However, we indicated in the present study that, immediate albumin administration had no significant depressing effect on VEGF expression in the hippocampus and cortex at 2 d, 3 d and 5 d after MCAO, VEGF expression in the cortex still remains at a high level at 2 d and 3 d after MCAO in the albumin group. On the basis of well documented results that VEGF administered 48-h post-ischemia significantly improves outcomes [29], we suggest that administration of albumin immediately followed by late VEGF therapy are not contradictory. Albumin administration possibly may not prevent VEGF from exerting its beneficial effects on brain vasculature later. Recent studies have demonstrated that tissue plasminogen activator (tPA) may aggravate ischemic neuronal damage after focal cerebral ischemia and increase BBB permeability. Combination therapy using tPA with albumin can attenuate the deleterious effects of tPA in acute ischemic stroke [8,21]. Therefore, we propose the combination therapy of using albumin immediately followed by late VEGF therapy may possibly produce better outcomes. Further studies are needed to evaluate whether they have a synergistic or additive effect.

The mechanism of how albumin administration affects VEGF expression remains unclear. Although the present study was not designed to investigate mechanisms of how albumin down-regulated VEGF expression in the early stage of ischemia, several possible links were proposed from literature review.

**Acknowledgments**

We deeply appreciate Professor James Braun in Tianjin Medical University for reviewing the manuscript. This work was supported by Tianjin Science & Technology Development Grant of China (No. 09JCYBJC11700) and Tianjin Educational & Scientific Grant (No. 20050107).

**References**


