Original Article

Protective effect of anti-intercellular adhesion molecule-1 antibody on global cerebral ischemia/reperfusion injury in the rat

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Summary **The present study aimed to clarify the protective effect of administration of an anti-intercellular adhesion molecule-1 (ICAM-1) antibody (1A29) on neurological damage after global cerebral ischemia/reperfusion in rats. Global cerebral ischemia/reperfusion was produced by four-vessel occlusion for 30 min followed by reperfusion for 24 h. Animals were randomly divided into four groups: PC group (n = 10), PI group (n = 10), PR group (n = 10), and PM group (n = 10). Rats in the PC group were administered isotype-matched control antibody at a dose of 1 mg/kg IV. Rats in the PI group, PR group, and PM group were infused with 1A29 at a dose of 1 mg/kg IV before ischemia, upon reperfusion, and 4 h into reperfusion, respectively. All animals were sacrificed after reperfusion for 24 h. Cerebral sections were stained with hematoxylin and eosin for histological evaluation. The brain wet-to-dry ratio and neurological deficits were evaluated. In comparison to the PC group, the counts of polymorphonuclear leukocytes (PMNLs) and macrophages (MΦ) decreased significantly in the PI, PR, and PM groups ($P < 0.01$). In comparison to the control antibody group, the brain wet-to-dry ratio and the percent infarct volume were significantly reduced in rats receiving 1A29 antibody ($P < 0.05$ and $P < 0.01$, respectively). In comparison to the PC group, with a median neurological score of 2.5, mild deficits were noted in the PI, PR, and PM groups (median neurological scores were 1.6 to 1.8) ($P < 0.05$). 1A29 antibody decreased the counts of PMNLs and MΦ and the neurological score and it reduced the brain wet-to-dry ratio and the infarct volume, suggesting that anti-ICAM-1 antibody provides neuroprotection after global cerebral ischemia/reperfusion injury in rats.**

Keywords: Global cerebral, ischemia/reperfusion, intercellular adhesion molecule-1, antibody, rat

1. Introduction

Inflammatory response and oxidative stress are known to exacerbate the damage caused by acute cerebral ischemia/reperfusion injury (*1,2*). Cytokines formed immediately after ischemia stimulate the expression of adhesion molecules on endothelial cells and leukocytes, leading to leukocyte adherence and extravasation into brain parenchyma (*3,4*). Extravasated polymorphonuclear leukocytes (PMNLs) release reactive oxygen species and lipid peroxidation products and promote blood-brain barrier disruption, vascular plugging, edema, and cerebral infarction (*5,6*).

The migration of leukocytes into injured tissue is regulated in part by a specific cell-surface integrin known as the CD18 receptor complex (*7*). Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein that is expressed on vascular endothelium and other cells. ICAM-1 expression facilitates leukocyte adhesion to endothelium (*8*).

Previous studies demonstrated that drugs designed to inhibit recruited leukocytes/microglia markedly curtailed inflammation and oxidative stress-related apoptosis and consequently provided neuroprotection in cerebral I/R injury (*9,10*). Previous studies showed a significant decrease in middle cerebral artery

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occlusion (MCAO)-induced brain damage in ICAM-1 knockout mice and anti-ICAM-1 antibody-treated rats (*11*). Treatment with the anti-ICAM-1 antibody reduces neurological deficits after spinal cord injury and embolic stroke in the rabbit (*12*). In rodents, hypothermic neuroprotection against focal ischemia is associated with attenuation of ICAM-1 induction and PMNL infiltration (*13,14*). Thus, these data support the hypothesis that neutrophils contribute to ischemic cell damage and blocking of ICAM-1 expression reduces ischemic cell damage. However, the optimal point in time for antibody administration has yet to be determined. The present study evaluated the efficacy of anti-ICAM-1 antibody used at various times to prevent infarct development and neurological deficiency after global cerebral ischemia/reperfusion injury in adult rats.

2. Material and Methods

2.1. *Animals*

Sprague-Dawley rats (weight, 180 to 200 g; $n = 40$) used in this study were cared for in accordance with the NIH guidelines for the ethical use of laboratory animals. The Research Animal Resources and Care Committee of Nanjing University approved the surgical procedures. Animals fasted for 8 h before surgery and were allowed free access to water. All animals were anesthetized with ketamine (80 mg/kg, i.p.).

2.2. *Four-vessel occlusion model*

A four-vessel occlusion model as described earlier was used to induce global cerebral ischemia/reperfusion (*14*). Under ketamine anesthesia, a pin 0.5 mm in diameter was inserted through each alar foramen and both vertebral arteries were cauterized and permanently occluded. Through a ventral mid-cervical incision, each carotid artery was isolated and a 9-0-nylon ligature was looped around it. After 24 h, global brain ischemia/reperfusion was induced by traction on both carotid ligatures for 30 min and then loosening of both ligatures. During ischemia/reperfusion, body temperature (37°C to 38°C) and cranial temperature (36°C to 37°C) were maintained at the physiological level.

2.3. *Grouping*

Animals were randomly divided into four groups: PC group ($n = 10$), PI group ($n = 10$), PR group ($n = 10$), and PM group ($n = 10$). Rats in all groups were subjected to ischemia for 30 min and reperfusion for 24 h. Rats in the PC group were administered isotype-matched control antibody at a dose of 1 mg/kg IV. Rats in the PI group were infused with 1A29 at a dose of 1 mg/kg IV before ischemia. Rats in the PR group and PM group were infused with 1A29 at the same dosage upon reperfusion and 4 h into reperfusion, respectively. Antibody to rat ICAM-1, designated 1A29 (*15*), reacts with the 85- to 89-kD epitope present on cytokine-activated rat endothelial cells. The endotoxin level of the anti-ICAM-1 antibody is less than 0.35 eu/mg. The control antibody has an endotoxin level of less than 1.0 eu/mg.

2.4. *Determination of degrees of brain injury*

All rats were sacrificed after reperfusion for 24 h. Tissues of the right cerebrum were processed and embedded in paraffin, and 4 μm-thick paraffin sections were stained with hematoxylin-and-eosin for histopathological evaluation. Six random high-double views (magnification, ×100) were taken to count PMNLs and macrophages (M*Φ*).

The volume of the ischemic lesion was computed by the numeric integration of data from 12 to 14 sections with respect to the sectional interval, as described earlier (*16*). The infarct volume was corrected to account for edema and shrinkage due to processing. The injury volumes were corrected using the following formula: corrected injury volume = contralateral hemisphere volume – (ipsilateral hemisphere volume – measured injury volume). The indirect method for calculating infarct volume, in which the intact area of the ipsilateral hemisphere was subtracted from the area of the contralateral hemisphere, was used. The infarct volume is presented as the percentage of the infarct lesion of the contralateral hemisphere.

2.5. *Measurement of the brain wet-to-dry ratio*

After the rats were sacrificed, cerebral tissues of the left hemisphere were removed and immediately weighed. The cerebral tissues were dried in an oven at 80°C for 12 h and reweighed. The brain wet-to-dry ratios were obtained by dividing the mass of the initial specimen by the mass of the dried specimen.

2.6. *Neurological evaluation*

Global cerebral ischemia/reperfusion–induced neurological deficit was evaluated on a 6-point scale following 1 day of reperfusion (before the animals were sacrificed) by an investigator blinded to the study groups, as described earlier (*17*). A score of 0 suggests no neurological deficit (normal), 1 suggests a mild neurological deficit (*e.g.* failure to fully extend the right forepaw), 2 suggests a moderate neurological deficit (*e.g.* circling to the right), 3 suggests a severe neurological deficit (*e.g.* falling to the right), and 4 suggests a very severe neurological deficit (*e.g.* failing to walk spontaneously and having a reduced level of consciousness).

2.7. *Statistical analysis*

All values are presented as mean ± standard error. Statistical evaluation was performed with the use of ANOVA followed by an unpaired *t* test. Significance was indicated by $P < 0.05$, and a high level of significance was indicated by $P < 0.01$.

3. Results

3.1. *Infiltration of PMNLs and MΦ*

Table 1 shows the PMNLs and MΦ counts in the 1A29 groups and the groups treated with control antibody. In comparison to the PC group, the counts of PMNLs and MΦ decreased significantly in the PI, PR, and PM groups ($P < 0.01$). No significant difference in the counts of PMNLs and MΦ was detected among the 1A29 groups.

3.2. *Wet-to-dry ratio of injured cerebral tissue*

Disruption of the blood-brain barrier was assessed by measuring water and fluid content in the brain. In comparison to the control antibody group, the brain wet-to-dry ratio was significantly reduced in rats receiving 1A29 antibody ($P < 0.05$) (Figure 1).

3.3. *Determination of infarct volume*

Table 2 shows representative infarct areas in rats with control antibody and 1A29 infusion. Total infarct volume did not differ significantly among the groups. In comparison to the control antibody group, the percent infarct volume decreased significantly in the 1A29-treated group after global cerebral ischemia/reperfusion injury ($P < 0.01$).

3.4. *Effect of anti-ICAM-1 antibody on neurological deficits*

The neurological deficits analyzed at 24-h reperfusion were severe in the PC group, with a median neurological score of 2.5, compared with mild deficits in the PI, PR, and PM groups (median neurological scores were 1.6 to 1.8) (Figure 2).

Table 1. PMNLs and MΦ infiltration on cerebral in various groups

Groups	Infiltration[a,b]	
	PMNLs	MΦ
PC	12.3 ± 2.5	2.1 ± 0.4
PI	5.3 ± 2 .0*	1.2 ± 0.3*
PR	6.0 ± 1.8*	1.1 ± 0.6*
PM	7.2 ± 2.4*	1.4 ± 0.2*

[a] Data are represented as the mean ± SE; [b] Comparison with PC group, * $P < 0.01$.

Table 2. Absolute hemisphere and lesion volumes and percent lesion volume of the contralateral hemisphere in various groups

Groups	Volumes (mm^3)[a,b]		% Lesion volume[b]
	Hemisphere	Lesion	
PC	438.7 ± 16.4	265.9 ± 7.8	60.6 ± 6.5
PI	425.1 ± 21.3	156.5 ± 11.7*	36.8 ± 4.8*
PR	429.9 ± 28.5	144.2 ± 12.4*	33.5 ± 7.5*
PM	434.5 ± 18.8	168.7 ± 9.6*	38.8 ± 3.9*

[a] Data are represented as the mean ± SE; [b] Comparison with PC group, * $P < 0.01$.

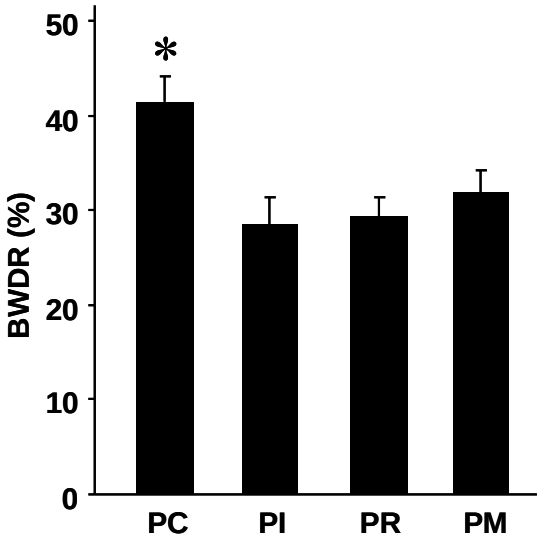

Figure 1. Brain water content in rats with global cerebral ischemia/reperfusion injury. Brain water content was determined by measuring the brain wet-to-dry ratio (BWDR) in rats with global cerebral ischemia/reperfusion injury. In comparison to the PC group, BWDR was significantly reduced in the PI, PR, and PM groups. * $P < 0.05$. Data were analyzed with the unpaired t test and are shown as mean ± SE.

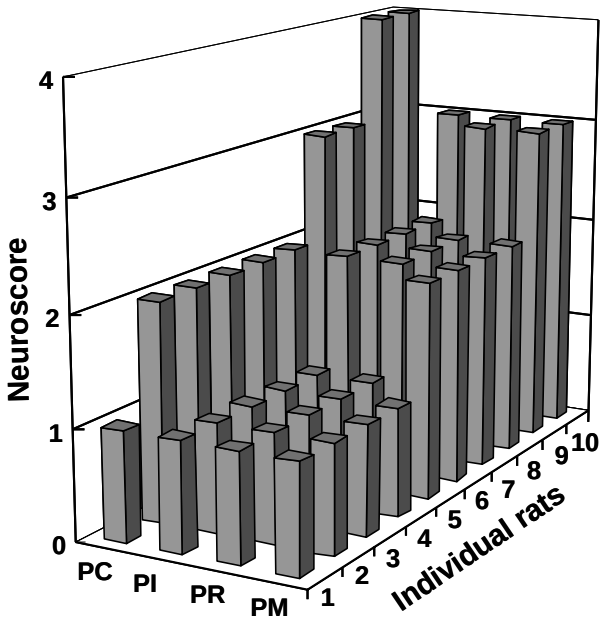

Figure 2. Individual neurological scores upon 24 h of reperfusion after global cerebral ischemia in rats with control antibody and rats with 1A29 infusion (n = 10 per group). In comparison to the PC group, with a median neurological score of 2.5, mild deficits were noted in the PI, PR, and PM groups (median neurological scores were 1.6 to 1.8). * $P < 0.05$.

4. Discussion

The current data indicate that intravenous administration of an anti-ICAM-1 antibody (1A29) significantly reduces the volume of infarcts and the brain wet-to-dry ratio and it decreases the subject's neurological score and the counts of PMNLs and MΦ. However, no significant difference in the above indices was observed among the 1A29 groups.

The protective effect of anti-ICAM-1 antibody against ischemic/reperfusion injury is attributed to the blockage of leukocyte adhesion, transendothelial migration, and improvement of blood flow during reperfusion (*18*). Ferulic acid provides neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting ICAM-1 mRNA expression in rats (*19*). Previous findings by the current authors revealed a new mechanism of hypothermia brain protection *via* inhibition of ICAM-1 expression and blocking of PMNL and MΦ infiltration in a rat global cerebral I/R injury model (*14)*. Studies have shown that prevention of ICAM-1 protein expression by antisense infusion significantly decreases transient focal ischemia–induced infarct size and neurological deficits (*20,21*). However, the choice of using antisense versus antibodies depends on the state of the patient. Antisense can be used as a preventive measure to bind to mRNA and inhibit ICAM-1 protein formation. Anti-ICAM-1 antibody may be more appropriate during ischemia/reperfusion injury by binding to ICAM-1 protein and halting its action.

The present study supports the contention that anti-ICAM-1 antibody administered either before ischemia or after reperfusion has a neuroprotective effect. The anti-ICAM-1 antibody is effective in reducing ischemic cell damage when administered during the reperfusion period and specifically 4 h after the initiation of reperfusion. This has positive implications for use of this form of therapeutic intervention in a clinical environment, where delayed intervention may be needed. Further study is required to determine the last point after reperfusion at which this therapy can be used.

In conclusion, the administration of the anti-ICAM-1 antibody significantly reduced global cerebral ischemic/reperfusion injury, regardless of whether it was before ischemia or after reperfusion.

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