Effects of Lipopolysaccharide Priming on Acute Ischemic Brain Injury

Shah-Hinan Ahmed, MD; Yong Y. He, MD; Abdullah Nassief, MD; Jian Xu, PhD; Xiao Ming Xu, PhD; Chung Y. Hsu, MD, PhD

Background and Purpose—Infection has been implicated as a stroke risk factor. Activation and infiltration of polymorphonuclear neutrophils (PMNs) after cerebral ischemia may contribute to ischemic brain injury. This study was conducted to investigate how enhanced postischemic PMN infiltration by lipopolysaccharide (LPS) altered the acute ischemic outcomes.

Methods—LPS (0.05 mg/kg SC) or vehicle was given to Long-Evans male rats 24 hours before ischemia. Focal cerebral ischemia was induced by temporary ligation of the right middle cerebral artery and both common carotid arteries for 45 minutes. Animals were killed 6 and 24 hours after reperfusion to determine the extent of PMN infiltration (myeloperoxidase assay), brain edema (wet-dry weight method), and vascular injury (fluorescein isothiocyanate–conjugated dextran extravasation). The infarct volumes were measured on the basis of TTC stain 24 hours after ischemia.

Results—LPS had little effect on body temperature or peripheral white count but substantially enhanced PMN infiltration into the ischemic right middle cerebral artery cortex on the basis of myeloperoxidase activity (6 hours: control, 0 U/g; LPS, 0.186±0.025 U/g; 24 hours: control, 0.185±0.025 U/g; LPS, 0.290±0.040 U/g; P<0.001) and morphological studies. The extent of vascular injury defined by the extravasation of fluorescein isothiocyanate–conjugated dextran into the ischemic tissue (6 hours: control, 3.11±0.41 μL/mg protein; LPS, 0.48±0.16 μL/mg protein; 24 hours: control, 1.77±0.23 μL/mg protein; LPS, 0.90±0.19 μL/mg protein; P<0.001) and brain edema determined by the brain water content (6 hours: control, 84.77±1.63%; LPS, 82.09±1.25%; 24 hours: control, 89.40±0.43%; LPS, 87.88±0.58%; P<0.01) were paradoxically reduced by LPS priming. LPS-primed rats also had smaller infarct volumes (control, 135±5 mm³; LPS, 108±12 mm³; P<0.05).

Conclusions—Enhanced postischemic PMN infiltration is anticipated to facilitate ischemic brain injury. Contrary to this expectation, results from the present study suggest that an increase in postischemic PMN infiltration after LPS priming was not detrimental. These findings challenge the notion that postischemic PMN infiltration is uniformly deleterious. (Stroke. 2000;31:193-199.)

Key Words: blood vessels ● brain edema ● cerebral ischemia ● inflammation ● neutrophils

Brain damage following cerebral ischemia/reperfusion may be accentuated by postischemic events, which constitute the secondary injury processes.1,2 Of the many pathophysiological events that may contribute to secondary injury, cell-mediated processes in the postischemic inflammation have been extensively reviewed.3-5 Key features of inflammation include vascular injury, edema formation, and infiltration of polymorphonuclear (PMN) and mononuclear leukocytes.5,6 PMNs are the initial primary effector leukocytes at the site of ischemia, entering 30 minutes after arterial occlusion and peaking at 24 to 48 hours, followed by other cells such as macrophages.7-9 The early accumulation of PMNs in the ischemic brain has been demonstrated by histopathological, biochemical, and 111In-labeled leukocyte studies.5-7 PMN infiltration is initiated after generation of inflammatory mediators such as C5a and leukotriene B4, expression of chemotactic cytokines10 and cell adhesion molecules (such as CD11/CD18 integrins, intercellular adhesion molecule-1 [ICAM-1], and P-selectin), and endothelial cell injury.11 PMNs may contribute to secondary injury after ischemia/reperfusion by causing microvessel occlusion12 and releasing oxygen radicals, cytolytic proteases, and proinflammatory cytokines,13 which, in addition to direct neuronal damage, may injure the endothelium. Reduction in the number of PMNs diminished postischemic tissue damage in the heart, intestine, lung, and liver.14 Recent therapeutic attempts

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to reduce PMN infiltration in animal stroke models have shown encouraging results. Various strategies, including inhibition of PMN activation and function by antineutrophil monoclonal antibody, 15 neutrophil inhibitory factor, 16 or interleukin-1 (IL-1) receptor antagonist17 and reduction of PMN adherence and trafficking by antibodies against adhesion molecules such as anti-CD11b/CD18 antibody14,18 and anti-ICAM antibody, 19 resulted in significant neuroprotective effects. Mice deficient in CD11b/CD18 also showed reduced ischemic injury after transient focal cerebral ischemia. 20 Leukocytes, particularly PMNs, have also been shown to accumulate in the infarcted region of human brains 2 to 14 days after ischemic stroke. 21 A study in stroke patients noted that intense PMN infiltration was correlated with massive tissue damage and poor neurological outcome. 22

Recent bacterial and viral infection, especially in the preceding week, has been implicated as a risk factor of ischemic stroke. 23 Activated PMNs in infection stimulate the recruitment and aggregation of platelets by releasing cathepsin G, a potent platelet agonist, 24 platelet activating factor, and leukotrienes. 25 This may activate the coagulation cascade or induce a procoagulant state during and after infection, leading to the development of acute ischemic stroke. 23 Lipopolysaccharide (LPS), an endotoxin released by gram-negative bacteria, enhances overall PMN reactivity. 26 In view of the reported detrimental role of PMN infiltration in cerebral ischemia/reperfusion and prior infection as a precipitating factor for stroke, we sought to further characterize the role of PMNs in ischemic brain injury by using LPS to enhance postischemic PMN infiltration in a stroke model featuring focal cerebral ischemia/reperfusion.

Materials and Methods
One hundred eight Long-Evans male rats (250 to 350 g body wt; Simonsen Laboratories, Gilroy, Calif) were used in this study. All procedures were approved by our institutional Animal Studies Committee and were in accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals, US Department of Agriculture Regulations, and the guidelines of the American Veterinary Medical Association Panel on Euthanasia. Rats were allowed free access to water and chow until surgery.

Stroke Model
The method for inducing severe ischemia in the cerebral cortex of the right middle cerebral artery (MCA) territory in rats has been described in detail. 27,28 In brief, Long-Evans male rats were anesthetized with injection of chloral hydrate (400 mg/kg IP). The right MCA was exposed by microsurgical techniques. A 2-mm burr hole was made at the junction of the zygomatic arch and squamous bone, allowing MCA ligation with the use of 10-0 suture. Both common carotid arteries (CCAs) were then occluded with nontraumatic aneurysm clips. In the present study, the duration of 3-vessel occlusion was 45 minutes. After ischemia, both the ligation on the right MCA and aneurysm clips on CCAs were removed. Before surgery, a blood sample was collected for peripheral white count. During ischemia and for 30 minutes after ischemia, body and left temporalis muscle temperatures were kept at 37±0.5°C with a heating lamp linked to thermostatic devices (Versa-Therm 2156, Cole-Parmer) and temperature probes, as described previously. 27 Arterial blood gases (Blood Gas Analyzer, model 238, Ciba Corning), mean arterial pressure (Digi-Med Blood Pressure Analyzer, Micro-Med, Inc), heart rate, and plasma glucose (Precision Glucose Analyzer, Medisense Inc) were also monitored before ischemia, during ischemia, and for 30 minutes after initiation of reperfusion. 28

After ischemia, all rats were kept in incubators ventilated at 24°C until the conclusion of the experiment 24 hours later.

Treatment With LPS
Animals were divided into LPS-primed and control groups. In the LPS group, rats were given a subcutaneous injection of LPS (Salmonella abortus equi, Sigma) at a dose of 0.05 mg/kg 24 hours before ischemia was induced. Preliminary studies with various doses of LPS showed that this dosing schedule did not cause apparent sickness or substantial alteration of physiological variables. The control group received vehicle injection 24 hours before ischemia.

Measurement of Myeloperoxidase
Myeloperoxidase (MPO) activity has been used to determine quantitatively the extent of PMN infiltration. 29,30 Six and 24 hours after ischemia, rats were perfused with 200 mL of saline intracardially under anesthesia. The right MCA cortex was dissected and stored at −70°C until assay for MPO. MPO assay has been previously described. 30 Briefly, the MCA cortex was homogenized in 0.05 mol/L potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. The supernatant derived from centrifugation at 27 000g for 15 minutes was assayed for MPO activity. MPO activity was measured spectrophotometrically in 2.9 mL of 0.05 mol/L phosphate buffer (pH 6.0) containing 0.53 nmol/L diaminoditylhydrochloride, 0.15 mmol/L H2O2, and 0.1 mL of the supernatant. One unit of MPO activity was defined as that degrading 1 μmol of H2O2 in 1 minute.

Histopathology
After perfusion with 200 mL of saline and 400 mL of 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), the brain was placed in the same fixative overnight and was embedded in paraffin. Coronal sections of the brain, 1 mm behind the bregma, were cut at 6 μm and stained with hematoxylin and eosin. Infarct areas in the LPS versus the control groups were searched for PMNs. The mature form of these cells has a lobulated and variably shaped nucleus and neutrophilic granular cytoplasm.

Determination of Extent of Vascular Injury
Increase in vascular permeability, a sensitive and specific indicator of vascular injury, is one of the important elements of an inflammatory response after an ischemic event. 31 The increase in vascular permeability was measured on the basis of the extent of extravasation of fluorescein isothiocyanate–conjugated dextran (FITC-D) (molecular weight, 71 200), as previously described. 32 Briefly, FITC-D (20 mg/kg) was administered by intravenous infusion 2 hours before the animals were killed by intracardiac perfusion with 200 mL of saline under anesthesia. The ischemic right MCA cortex was then dissected and homogenized in 1.0 mL of 5% trichloroacetic acid and centrifuged at 27 000g for 20 minutes. A 0.8-mL aliquot of the supernatant was mixed with 0.2 mol/L Tris buffer (pH 8.2) to measure the FITC-D fluorescence intensity at 490 nm for excitation and 521 nm for emission. A sample of blood was obtained from the heart before perfusion. For the assay of FITC-D levels in plasma, 10 μL of plasma was mixed with 1.0 mL of 5% trichloracetic acid. After centrifugation, 0.8 mL of the supernatant was added to 0.2 mol/L Tris buffer (pH 8.2), and FITC-D fluorescence was determined with the use of the same procedure for the brain. The extent of vascular injury was estimated by a vascular injury index (VII), 32 derived from the following formula:

\[
\text{VII} = \frac{\text{FITC-D in Right MCA Cortex} - \text{Protein Content in Right MCA Cortex (mg)}}{\text{Plasma FITC-D Concentration/Plasma (μL)}}
\]

Measurement of Brain Edema
Determination of the extent of brain edema by the wet-dry method has been described previously. 33 The ischemic MCA cortex was removed in a humidity chamber, and the wet weight was measured
immediately. The ischemic cortex was dried at 100°C to a constant weight for the determination of dry weight. Brain edema was determined by calculating tissue water content according to the following formula: Percentage of Brain Water Content = \( \frac{100}{(1 - \text{Dry Weight/Wet Weight})} \).

**Morphometric Analysis of Infarct Volume**

Infarct areas were demarcated by 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma) staining, and infarct volumes were calculated by the "indirect" morphometric analysis method. In brief, animals were killed with an overdose of pentobarbital (100 mg/kg IP), and brains were sliced into 2-mm coronal sections with the use of a brain matrix (Harvard Bioscience). The brain sections were incubated in phosphate-buffered saline containing 2% TTC at 37°C for 20 minutes and subsequently stored in 10% phosphate-buffered formalin. The infarct area of each brain slice was determined with an image analyzer (DUMAS, Drexel University). The volume of the infarct was calculated by summing the infarct areas measured in the component brain slices. TTC-defined infarct volumes correlated with those measured by a conventional histological method with the use of hematoxylin and eosin stain. The morphometric analysis of infarct volume, in which the indirect method was used to correct for biases caused by brain edema, was described in detail elsewhere.

**Statistical Analysis**

Data are expressed as mean±SD. Multiple samples were analyzed by 1-way ANOVA followed by a post hoc Tukey test corrected for multiple comparisons. Comparisons of changes in variables over time were made with the use of 2-way ANOVA. Differences between 2 groups (LPS primed versus control) were analyzed by Student’s t test. A P value <0.05 was considered significant.

**Results**

The LPS dose (0.05 mg/kg), chosen in a preliminary study, was found to be without serious systemic effects on the animals. There was a slight difference in core temperature between groups before and after ischemia, with LPS-primed rats showing slightly higher core temperature at 6 hours after LPS administration and at 2 and 6 hours after surgery (Figure 1). The peripheral white cell counts were also slightly higher in LPS-primed rats than in vehicle-treated animals (control, 13 033 ± 776/mm³, n = 3; LPS, 15 272 ± 1956/mm³, n = 4). The difference was not significant. Differences in physiological variables were also assessed between LPS and control groups. The preischemic, intraischemic, and postischemic heart rate, mean arterial pressure, blood gases, and plasma glucose levels were not different between LPS and control groups (Table 1). The plasma levels of cytokines such as tumor necrosis factor-α (TNF-α) or IL-1 were not increased with this dose of LPS (data not shown). MPO activity in the brain was measured to assess the extent of PMN infiltration in the

**Figure 1.** Effect of LPS on core temperature before and after ischemia. Core temperature was measured in LPS- and vehicle-treated rats before and after LPS or vehicle administration and for 24 hours after ischemia. Note slight but significantly higher core temperature in LPS-primed animals at several times. *Difference from control is significant.

**TABLE 1. Physiological Variables**

<table>
<thead>
<tr>
<th></th>
<th>Before MCA Occlusion</th>
<th>During MCA Occlusion</th>
<th>After MCA Occlusion</th>
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<tbody>
<tr>
<td><strong>Blood pressure, mm Hg</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control (n=12)</td>
<td>86.7±7.8</td>
<td>86.5±9.1</td>
<td>90.4±11.6</td>
</tr>
<tr>
<td>LPS (n=4)</td>
<td>83.8±7.5</td>
<td>88.7±2.5</td>
<td>83.7±6.3</td>
</tr>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>407.3±45.0</td>
<td>380.0±65.4</td>
<td>384.6±50.1</td>
</tr>
<tr>
<td>LPS (n=4)</td>
<td>400.0±23.1</td>
<td>420.0±49.0</td>
<td>380.0±42.4</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
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<tr>
<td>Control (n=11)</td>
<td>7.34±0.02</td>
<td>7.37±0.05</td>
<td>7.35±0.03</td>
</tr>
<tr>
<td>LPS (n=4)</td>
<td>7.35±0.03</td>
<td>7.36±0.02</td>
<td>7.33±0.03</td>
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<td><strong>Paco₂, mm Hg</strong></td>
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<tr>
<td>Control (n=11)</td>
<td>40.3±7.9</td>
<td>36.0±7.4</td>
<td>33.5±7.3</td>
</tr>
<tr>
<td>LPS (n=4)</td>
<td>35.8±1.5</td>
<td>39.5±12.1</td>
<td>44.5±6.4</td>
</tr>
<tr>
<td><strong>Pao₂, mm Hg</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Control (n=11)</td>
<td>81.5±9.5</td>
<td>93.0±15.2</td>
<td>92.7±8.0</td>
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<tr>
<td>LPS (n=4)</td>
<td>82.8±14.8</td>
<td>77.2±14.4</td>
<td>86.5±7.1</td>
</tr>
<tr>
<td><strong>Plasma glucose, mg/dL</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Control (n=6)</td>
<td>152.3±59.0</td>
<td>181.8±38.2</td>
<td>139.2±22.6</td>
</tr>
<tr>
<td>LPS (n=6)</td>
<td>131.2±60.5</td>
<td>173.3±27.6</td>
<td>144.0±19.0</td>
</tr>
</tbody>
</table>
ischemic cortex. The sensitivity of MPO assay is $5 \pm 1 \times 10^{-3}$ U/min.\textsuperscript{10} MPO activity was significantly higher at both 6 and 24 hours after ischemia in the LPS group than in the control group (Figure 2). Thus, LPS priming resulted in a substantial increase in the extent of PMN infiltration in the ischemic cortex. The increase in PMN infiltration based on MPO assay was further confirmed by morphological studies. A well-defined area of infarction was noted in the right MCA cortex. In the control group, a few scattered PMNs were noted in the ischemic right MCA cortex. In contrast, numerous PMNs were seen infiltrating the area of infarction in LPS-primed rats (Figure 3). Using the FITC-D method to assess the extent of vascular injury, we noted that the VII was lower in LPS-primed rats than in controls at 6 and 24 hours (Table 2). LPS pretreatment resulted in significant reduction in vascular permeability at both times. The brain water content, a measure of the extent of edema formation based on the wet-dry weight method, was also assessed at 6 and 24 hours after ischemia. The brain water content in the MCA cortex was significantly lower in the LPS than in the control group at both times (Table 2). Infarct volumes as determined by TTC staining were significantly smaller in the LPS than in the control group (Table 2).

**Discussion**

Inflammatory events are thought to contribute to the secondary injury process after ischemia/reperfusion.\textsuperscript{1–5} The pathogenetic role of PMNs in ischemic injury has already been extensively studied. Recent therapeutic attempts to reduce PMN activation and infiltration into the ischemic brain in animal stroke models have shown encouraging results in improving stroke outcomes.\textsuperscript{13–20} Collectively, these studies suggest the deleterious effects of PMNs in the setting of acute focal cerebral ischemia.

The present study was undertaken to determine whether LPS priming enhances postischemic PMN infiltration and consequently accentuates acute ischemic brain injury, assuming a deleterious role of PMNs in focal cerebral ischemia. LPS is a pluripotent activator of PMNs. LPS stimulates...
PMNs in various ways, including enhancement of PMN adherence to endothelial cells. LPS also primes PMNs to release superoxide ($O_2^-$) in response to other stimuli. Its lipid A part activates the complement C5a, which is a potent chemoattractant for PMNs. LPS priming leads to increased production of the acute phase reactants (IL-1 and TNF-$\alpha$) and leukotriene $B_4$, which are also chemoattractants for PMNs. LPS (0.05 mg/kg) given subcutaneously 24 hours before ischemia was chosen from a series of preliminary studies to avoid inflammatory reactions including fever, leukocytosis, hypotension, and other serious systemic effects that may confound ischemic outcomes. There was no substantial alteration of body temperature or increase in peripheral white counts in the LPS-primed rats compared with the controls. The plasma levels of TNF-$\alpha$ or IL-1 were not elevated with this dose of LPS. Arterial blood gases, heart rate, mean arterial pressure, and plasma glucose were also not significantly altered in the LPS group. These findings indicate that the dose of LPS (0.05 mg/kg) used to prime PMNs did not itself cause a substantial inflammatory reaction or cardiovascular dysfunction in rats that were subjected to focal cerebral ischemia 24 hours later. However, a single dose of LPS apparently primed PMNs for $>24$ hours, leading to an enhanced infiltration into the ischemic cortex, as reflected by the substantial increase in MPO activity. MPO assay is a quantitative measure of the extent of PMN infiltration. Other inflammatory cells in brain, such as monocytes and activated microglia/macrophages, may also contain this enzyme, but to a much lesser extent. Furthermore, the mononuclear cells represent only a very small percentage of activated cells at the inflammation site in the early stage of postischemic inflammation reaction. Very few monocytes or microglia/macrophages were noted on morphological examination in the present study. However, a slight contribution of these mononuclear cells to the MPO assay cannot be completely ruled out.

Knowing the possible contributions of PMNs to the secondary injury processes after ischemia, we had expected that an increase in PMNs at the ischemic site would lead to greater acute brain injury. The fact that infection in the preceding week is a major risk factor for ischemic stroke also suggests that enhanced PMN activity may be detrimental in the setting of focal cerebral ischemia/reperfusion. Clinical studies have also suggested a positive correlation between activated PMNs and exacerbation of acute ischemic injury in stroke patients. Unexpectedly, we noted a significant reduction in vascular injury reflected by the extent of FITC-D extravasation. The brain water content and infarct volumes were also significantly reduced in the LPS group, in which the postischemic PMN infiltration in the ischemic cortex was greatly enhanced. These findings contradict a notion that an increase in PMN infiltration in the ischemic brain is invariably associated with a greater extent of tissue damage. It should be noted that the role of PMNs in acute ischemic brain injury has not been clearly delineated. Certain therapeutic strategies directed at reducing PMN infiltration or inhibiting PMN activation have failed in selected stroke models. A recently concluded double-blinded, randomized, placebo-controlled trial also showed that anti–ICAM-1 antibody was not effective and might even be detrimental in patients with acute ischemic stroke. Results from an animal study have also weakened the likelihood that infiltrating PMNs are invariably detrimental in ischemia/reperfusion injury.

Preconditioning with LPS seemed to have an overall protective effect on the ischemic brain. LPS priming that increased tolerance against focal cerebral ischemic insult could be due to its stimulation of TNF-$\alpha$ expression, since TNF-$\alpha$ has also been shown to confer protection against focal cerebral ischemia. However, LPS and TNF-$\alpha$ could be deleterious, exacerbating ischemic and hemorrhagic lesions in rats with inherent stroke risk factors. These observations suggest that effects of LPS priming may be variable depending on the physiological states and the presence of certain risk factors for vascular injury. Other possible mechanisms of LPS-induced tolerance against ischemia could be due to increased production of nitric oxide, upregulation of antioxidants, or de novo synthesis of protective proteins independent of TNF-$\alpha$. LPS pretreatment does not seem to affect the cerebral blood flow immediately after MCA occlusion but may diminish the severity of secondary microvascular perfusion deficits.

In conclusion, the present study shows that an increase in postischemic PMN infiltration under a selected experimental paradigm did not accentuate acute ischemic brain injury. These findings are compatible with the notion that PMN infiltration into the ischemic brain may not be uniformly deleterious. Further studies are needed to fully explore the complex roles of PMNs in the setting of focal cerebral ischemia/reperfusion. It is conceivable that LPS priming may exert a preconditioning protective effect. Whether this LPS effect involves TNF-$\alpha$ or other mechanisms remains to be elucidated.

**Acknowledgments**

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**TABLE 2. Effect of LPS on Vascular Permeability, Brain Edema, and Infarct Volume**

<table>
<thead>
<tr>
<th>Time After Ischemia, h</th>
<th>Control</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIL, $\mu$L/mg protein</td>
<td>6</td>
<td>3.11±0.41 (n=10)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.77±0.23 (n=7)</td>
</tr>
<tr>
<td>Water content, % of brain weight</td>
<td>6</td>
<td>84.77±1.63 (n=5)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>89.40±0.43 (n=6)</td>
</tr>
<tr>
<td>Infarct volume, mm$^3$</td>
<td>24</td>
<td>135±5 (n=6)</td>
</tr>
</tbody>
</table>

*Difference from controls is significant (P<0.05).
References


LPS (or bacterial endotoxin) is known to have a variety of effects on the brain and cerebral circulation. At the molecular level, these changes include activation of transcription factors, changes in gene expression, formation of proinflammatory and anti-inflammatory cytokines, expression of adhesion molecules, and infiltration of leukocytes. Many of these same changes, including the infiltration of leukocytes, occur following cerebral ischemia and are generally thought to contribute to neuronal dysfunction and cell death.1

The goal of this study was to further examine the role of leukocytes in brain injury following ischemia using LPS in a rat model of focal ischemia. As part of the protocol, a relatively low dose of LPS was administered 24 hours before ischemia. The results suggest that pretreatment or “preconditioning” with a low dose of LPS 24 hours before ischemia produces increased infiltration of PMNs in the ischemia brain. In contrast to what probably would have been predicted on the basis of previous studies which suggested that infiltration of leukocytes contributes to brain injury after ischemia,1 this increased leukocyte infiltration was associated with neuroprotection (reduced edema and infarct volume). These findings suggest that, at least under some conditions, increased infiltration of PMNs does not contribute to brain injury.

One limitation of the present experiment is that it does not provide insight into the mechanism by which pretreatment with LPS exerts this protective effect in this model. What are some possibilities? There are several levels at which one might hypothesize that LPS preconditioning could exert protective effects for cerebral ischemia, including the reprogramming of gene expression such that subsequent injury in response to ischemia is limited. There is precedent for such reprogramming of gene expression in the heart, where LPS pretreatment produced increased expression of manganese superoxide dismutase and catale (scavengers of reactive oxygen species).2

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