Toll-Like Receptor 4-Mediated Myeloid Differentiation Factor 88-Dependent Signaling Pathway Is Activated by Cerebral Ischemia-Reperfusion in Hippocampal CA1 Region in Mice

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The Toll-like receptor 4 (TLR4)-mediated myeloid differentiation factor 88 (MyD88)-dependent signaling pathway plays an essential role in inflammation resulting from invading microbes. However, whether the signaling pathway is activated in the inflammatory reaction of cerebral ischemia-reperfusion and its mechanism is still unclear. In this experiment mice were randomly divided into sham group, ischemia/reperfusion group and TLR4-blocked group with different time points of reperfusion at 12, 24, 48 and 72 h. Mice cerebral ischemia was induced by occlusion of common carotid arteries (CCA) bilaterally. TLR4 signaling pathway was inhibited using specific anti-TLR4 binding protein to prevent TLR4 from interacting with its receptors. We determined the result of TLR4 antibodies-blocking and mice cerebral ischemia-reperfusion injuries by Western blot, and evaluated neuronal damage in the hippocampus. We also determined expression of TLR4 mRNA and MyD88 mRNA by in situ hybridization (ISH), activation of nuclear factor (NF)-κB by electrophoretic mobility-shift analysis (EMSA), and expression of interleukin (IL)-1β protein by Western blot. The results demonstrated that TLR4-mediated MyD88-dependent signaling pathway activated by ischemia-reperfusion may be involved in the mechanism of ischemia-reperfusion through upregulation of NF-κB, IL-1β.

Key words Toll-like receptor 4; ischemia; reperfusion; antibodies-blocking

The membrane-bound Toll-like receptor 4 (TLR4) is a transmembrane receptor with a cytoplasm Toll/interleukin-1 receptor (TIR) homology domain. TLR4-mediated MyD88-dependent signaling pathway plays an essential role in detection and elimination of invading microbes. The TIR domain can interact with the adapter protein myeloid differentiation factor 88 (MyD88). Upon stimulation, TLR4 triggers the signaling pathway, which culminates in activation of the transcription factor nuclear factor-κB (NF-κB), and results in expression of genes involved in innate and inflammatory responses including the production of pro-inflammatory cytokines such as IL-1β. Studies have shown that innate immune and inflammatory responses play a critical role in cerebral ischemia-reperfusion injury. Recent evidence suggests that TLR4-NF-κB signaling contributes to myocardial ischemia-reperfusion injury. Considering the role of TLR4-mediated MyD88-dependent signaling pathway, we infer that TLR4 signaling pathway may be involved in the damage of cerebral ischemia-reperfusion. Little is known about this aspect. To investigate whether TLR4 signaling pathway is activated by ischemia-reperfusion, we examined the expression of TLR4 mRNA and MyD88 mRNA, activation of NF-κB and expression of IL-1β protein in the CA1 area of mice hippocampus which is the most vulnerable to ischemia. We used the TLR4-blocked model in mice. TLR4 signaling pathway can be inhibited using specific TLR4 antibodies (anti-TLR4) binding protein to prevent TLR4 from interacting with its receptors.

MATERIALS AND METHODS

Chemicals and Reagents The following primary antibodies were used: rabbit polyclonal anti-IL-1β and monoclonal anti-TLR4 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). EMSA kit and horseradish peroxides (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) were also obtained from Santa Cruz Biotechnology. ISH kit was purchased from Nippon Gene Biotechnology (Nippon Gene, Toyama, Japan). Antibody against β-actin was purchased from Sigma. TUNEL kit was purchased from Roche. All other reagents were purchased from Sigma unless otherwise mentioned.

Experimental Animals Healthy Kunming mice (8 weeks old, weight 18—22 g) were supplied by the Experimental Animal Center of China Medical University. All mice expressing normal functional TLR4 were bred in our laboratory, and kept in-house under pathogen-free condition. The animal procedures were approved by the Animal Care Use Committee of China Medical University (SYSC 2003—009). The mice in the present study were randomly divided into three groups: sham (S group, n=72), ischemia/reperfusion (I group, n=72) and TLR4-blocked (T group, n=72) with different time points of reperfusion at 12, 24, 48 and 72 h (n=18/group).

Animal Surgical Procedures Animal surgical procedures were performed according to the strict sterility standard. Animals were anesthetized intraperitoneally with 2% pentobarbital sodium (40 mg/kg) (Forene, Abbott GmbH, Wiesbaden, Germany). Cerebral ischemia/reperfusion was induced by a previously described method. Briefly, cerebral ischemia was induced by occlusion of common carotid arteries (CCA) bilaterally. The CCA were gently isolated and clamped with microsurgical clamps. Cerebral ischemia was maintained for 12 min and reperfusion started when the clamps were removed. We kept the temperature at about 25 °C during and after surgery, exposed animals in incandes-
cent lamp to keep their rectal temperature at about 37°C until palinesthesia (Mini Vent 845, Hugo SachsElektronik, March-Hugstetten, Germany). The ischemic condition and sufficiency of reperfusion were confirmed by cerebral blood flow detected by Laser Doppler Perfusion Monitor (BS EN 60825-1, U.K.). Two minutes before reperfusion, anti-TLR4 (10 μg/ml) was injected into the right CCA using a microringe (B/BRAUN8714843, Germany) over 2 min for blocking TLR4 receptors while 0.9% NaCl was injected into the right CCA of I group animals with the same dose and method. The mice were placed in cages at 25°C for the following 3 h then returned to the animal care room. Sham animals received the same surgical procedures except for occluding common carotid arteries bilaterally and injecting anti-TLR4. Each group of mice (n=12) was sacrificed by pentobarbital sodium overdose at 12, 24, 48 and 72 h, respectively. The brains were removed and stored at −80°C for isolation of cellular proteins. Nuclear extracts of the right hippocampal tissues were used for analysis of NF-κB activation by electrophoretic mobility-shift assay (EMSA). Cellular proteins of the right hippocampal tissues were used for analysis of IL-1β protein levels and the result of TLR4-block by Western blot. At 12, 24, 48 and 72 h reperfusion, each group of mice (n=6) was anesthetized intraperitoneally with 2% pentobarbital sodium and transcardially perfused with saline followed by 4% paraformaldehyde in phosphate buffered saline (PBS) (0.1 mol/l, pH 7.4) and post-fixed.11 The brains were removed, embedded in paraffin and cut into sections (7 μm). The right hippocampal formation slides were detected by in situ hybridization for analysis of TLR4 mRNA and MyD88 mRNA levels and evaluation of neuronal damage in the hippocampus by hematoxylin–eosin (H–E) staining.

Evaluation of Neuronal Damage in the Hippocampus
Since the CA1 area of the hippocampus is the region most vulnerable to ischemia-reperfusion injury, we analyzed this region for neuronal damage. According to the stereotaxic mouse brain atlas, we took out the hippocampal tissues. The CA1 area of the hippocampus is the region most vulnerable to ischemia-reperfusion injury, we analyzed this region using a defined rectangular field area with magnification 400X.

TLR4 Antibodies-Blocking TLR4 antibodies-blocking was performed as described previously.9 Two minutes before reperfusion, anti-TLR4 (10 μg/ml) was injected into the right CCA of T group animals. The result of blocking was detected by Western blot analysis.

In Situ Hybridization (ISH) The dioxigenin (DIG)-labeled mRNA probe for TLR4 and MyD88 was obtained from Pub Med and purchased from Haoyang Bio. (Tianjin, China). The sequences of TLR4 mRNA probe and MyD88 probe were: 5′-TCAGAATAAGAACAGCAACCACATTAAACG-C3′ and: 5′-GCTTCTCGACTCCTGTTTCGTGCTTA-3′, respectively. ISH was performed as described previously.13 Because the CA1 area of HF is the region most vulnerable to ischemia-reperfusion, we analyzed the TLR4 mRNA and MyD88 mRNA levels in this region. Paraffin-embedded specimens were sliced at a 7 μm thickness. These sections were mounted on poly-l-lysine-treated (Vector Lab, U.S.A.) slides, deparaffinized and rehydrated. The sections were blocked by immersion in H2O2 at room temperature for 20 min. They were further digested with protease K 1 μg/ml, pepsin 20 μg/ml, ethylenediamine tetraacetic acid (EDTA) 0.1 mg/ml, pH 6.4 at room temperature for 30 min, then incubated in prehybridization solution at 37°C for 1 h in a humidified chamber. Slides were washed thrice in 0.2×SSC (50 mM NaCl, 3 mM Na citrate), 5 min each. For hybridization, each slide was incubated in hybridization solution (8 μg/ml) at 37°C for 4 h in a humidified chamber, then sections were washed thrice in 0.2×SSC, 2×SSC, 0.1 mol TBS (NaCl 8.5 mg/ml, Tris 1.2 mg/ml, pH 7.5), for 5 min each. Each slide was carried out by diluted solution containing the anti-DIG (DB7405), then was subjected to the solution containing the high sensitive peroxidase–chain–avidin at 37°C for 45 min in a humidified chamber. The mean optical density (MOD) of positive cells was evaluated by Motic Images Advanced 3.2.

Electrophoretic Mobility Shift Assay (EMSA) Preparation of nuclear extracts and gel mobility shift assays were performed according to methods described previously.14 The sequence of the NF-κB-binding oligonucleotide used as a Biotin-probe was 5′-TCGACAGAGGGACTTTCCGAGGCCGTTGACAGAGGGACTTTCCGAGGCCGTCAGAATAAGAACAGCAACCACATTAAACG-C3′. Equal amounts of nuclear extracts (7 μg) from right hippocampus in each group at each time point were incubated with Binding Reaction [binding buffer 1.5 μl, Poly(dI:dC) 0.5 μl, dhO2, biotin-probe 0.5 μl] at room temperature for 20 min, then were electrophoresed on 6.5% polyacrylamide gel (40% acrylamide/Bis 3.3 ml, 50% glyceral 1.0 ml, dhO2, tetramethylethylenediamine (TEMED) 20 μl) on ice at 180 V for 60 min. Competition was performed by adding 3.3 μl unlabeled DNA along with the biotin-probe. The DNA–protein complexes and unbound probe were separated electrophoretically on 6.5% polyacrylamide gel in 0.5×TBE buffer. The membrane was blocked with 15 ml blocking buffer for 30 min. Chemiluminescence was developed by the system of Chemiluminecence-imager (Chemi Imager 5500, U.S.A.). The membrane was exposed to X-ray film at room temperature for 3—5 min. NF-κB activity was examined by the integrated density value (IDV) of the band.

Immunohistochemistry Staining for NF-κB The nuclear translocation of NF-κB was detected in mice right hippocampal CA1 region at ischemia-reperfusion 12, 24, 48 and 72 h by immunohistochemistry using specific anti-NF-κB antibody. Brains containing hippocampal tissues from each group were harvested and immersion-fixed in 4% buffered paraformaldehyde, embedded in paraffin, cut at 7 μm, and stained with an antibody directed against activated NF-κB. Five slides from each block were evaluated with brightfield microscopy.
Western Blot for IL-1β Western blot was performed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) Bis-Tris gel electrophoresis as described previously. For total cellular protein, cells were lysed in buffer containing 25 mM Hepes, pH 7.5, 0.3 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.1% Triton X-100, 20 mM β-glycerophosphate, 0.5 mM dichlorodiphenyl trichloroethane (DDT), 1 mM sodium orthovanadate, 0.1 μM okadaic acid, and 1 mM phenylmethylsulfonyl fluoride. Protein concentrations of the cell lysates were determined by Lowry method with bovine serum albumin as standard, and the supernatants were boiled in SDS sample buffer for 5 min. Equal amounts of lysate protein were run on 12% SDS-PAGE and electrophoretically transferred to PVDF membrane (Amersham-Pharmacia Biotech). After blocking, the blots were incubated with specific primary antibody at 4 °C overnight and further incubated for 1 h with HRP-conjugated secondary antibody. Bound antibodies were detected by ECL kit with a Lumino Image Analyzer (TAITEC).

Statistical Analysis The IDV of Western blot and EMSA band and the MOD of ISH section were evaluated by means of the Motic Images Advanced 3.2 (BX51 + PM10SP-35, OLYMPUS, Japan). Values are expressed as means ± standard deviation (X ± S.D.). All data were analyzed by SPSS13.0 software. For tests of significance between groups, one-way analysis of variance (ANOVA) was performed. Significance was regarded as p < 0.05.

RESULTS

Western Blot for TLR4 Antibodies-Blocking To evaluate the result of TLR4 antibodies-blocking, Western blot analysis was carried out to study the TLR4 protein levels in each group. Figure 1A shows that the protein levels of TLR4 in I group were obviously higher than those in T group and S group. The IDV of TLR4 in I group was higher than that in T group at 12, 24, 48 and 72 h (p < 0.05) (Fig. 1B). In the present study, the results demonstrated that the protein levels of TLR4 in I group were expressed excessively and anti-TLR4 antibody binding TLR4 receptors before reperfusion was effective.

Hematoxylin–Eosin (H–E) for Evaluation of Neuronal Damage in the Hippocampus We observed the tissue sections of hippocampal CA1 region and assessed cerebral histological changes of mice caused by ischemia-reperfusion under light microscopy. Neuronal damage was evaluated qualitatively by a pathologist blinded to the treatment groups. In the S group, although neurons were arranged orderly and intact in shape, they were swollen, with shrunken cell bodies, triangulated, pyknotic, the cellular inter-space widened and arranged asymmetrically in I group, while obviously palliated in T group (p < 0.05). The evident changes in I group were found 24 h after ischemia-reperfusion, reaching maximum at 48 h, then decreasing gradually at 72 h (Fig. 2, Table 1). The positive products of TUNEL staining were brown.

Fig. 1. Expression of TLR4 Protein in Each Group in Mice Right Hippocampal CA1 Region at Ischemia-Reperfusion 12, 24, 48 and 72 h by Western blot (A) and the IDV of TLR4 in Each Group with Different Reperfusion Time Point (B)

Data are expressed as mean ± S.D. from six animals. ∗p < 0.05 compared with S group or T group.

Fig. 2. Sections of 4% Paraformaldehyde-Fixed, Paraffin-Embedded Right Hippocampal Formation Were Stained with Hematoxylin–Eosin (H–E) and TUNEL Methods to Reveal CA1 Region
A representative histology section of 48 h after ischemia-reperfusion in I group and normal neurons in S group are shown, while the above changes were obviously palliated in T group (n = 6) by (H–E) method. The positive products 48 h after ischemia-reperfusion showed brown and mainly expressed in right hippocampal CA1 regions in I group, while the above changes were obviously palliated in T group (n = 6) by TUNEL method. Bar = 25 μm.
particles. The number of apoptotic cells was increased when ischemia-reperfusion was lengthened and reached peak at 48 h. At the same time point, the number of apoptotic cells was more than that of S group, and the number of apoptotic cells of T group was less than that of I group (Fig. 2, Table 2).

Expression of TLR4 mRNA and MyD88 mRNA by in Situ Hybridization (ISH) To investigate whether TLR4 and MyD88 are activated by ischemia-reperfusion, we examined the expression of TLR4 mRNA and MyD88 mRNA in each group at each time point after ischemia-reperfusion. Expression of TLR4 mRNA and MyD88 mRNA was determined by in situ hybridization (ISH) method. There was apparent expression of TLR4 mRNA and MyD88 mRNA in right hippocampal CA1 region in I group and T group. The positive products for TLR4 mRNA showed brown, and mainly expressed in cytoplasm. TLR4 mRNA positive products in right hippocampal CA1 regions in different groups after ischemia-reperfusion at 12, 24, 48 and 72 h (A). MOD of positive cells for TLR4 mRNA in each group (B). Data are expressed as mean±S.D. from six animals. Bar=25 μm. *p<0.05 compared with S group or T group.

Expression of MyD88 mRNA by in Situ Hybridization (ISH) in Right Hippocampal CA1 Region in Each Group

The positive products for MyD88 mRNA showed brown, and mainly expressed in cytoplasm. MyD88 mRNA positive products in right hippocampal CA1 regions in different groups after ischemia-reperfusion at 12, 24, 48 and 72 h (A). MOD of positive cells for MyD88 mRNA in each group (B). Data are expressed as mean±S.D. from six animals. Bar=25 μm. *p<0.05 compared with S group or T group.

Table 1. Neuropathological Score Following Cerebral Ischemia-Reperfusion in Each Group by (H–E) Method

<table>
<thead>
<tr>
<th>Reperfusion time (h)</th>
<th>I/R</th>
<th>TLR4 block</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1.2±0.32*</td>
<td>0.4±0.16</td>
<td>0.1±0.08</td>
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<tr>
<td>24</td>
<td>2.1±0.32*</td>
<td>1.3±0.09</td>
<td>0.1±0.06</td>
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<tr>
<td>48</td>
<td>3.3±0.35*</td>
<td>1.6±0.16</td>
<td>0.1±0.03</td>
</tr>
<tr>
<td>72</td>
<td>2.8±0.06*</td>
<td>1.4±0.15</td>
<td>0.1±0.04</td>
</tr>
</tbody>
</table>

*p<0.05 vs. sham or TLR4 block.

Table 2. No. Apoptotic Cells Following Cerebral Ischemia-Reperfusion in Each Group by TUNEL Method

<table>
<thead>
<tr>
<th>Reperfusion time (h)</th>
<th>I/R</th>
<th>TLR4 block</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>71.09±25.33*</td>
<td>45.23±11.27</td>
<td>32.94±15.26</td>
</tr>
<tr>
<td>24</td>
<td>91.64±20.18*</td>
<td>44.38±15.84</td>
<td>33.60±8.12</td>
</tr>
<tr>
<td>48</td>
<td>111.29±24.96*</td>
<td>50.28±19.37</td>
<td>35.13±15.96</td>
</tr>
<tr>
<td>72</td>
<td>95.42±31.40*</td>
<td>41.77±18.16</td>
<td>35.05±19.28</td>
</tr>
</tbody>
</table>

*p<0.05 vs. sham or TLR4 block.
ually at 72 h (Fig. 3A). Expression of MyD88 mRNA showed the similar result (Fig. 4A). The mean optical density values (MOD) of positive cells for TLR4 mRNA in T group were lower than those in I group ($p<0.05$) (Fig. 3B). The MOD of positive cells for MyD88 mRNA in T group was lower than that in I group ($p<0.05$) (Fig. 4B). Both MOD of TLR4 mRNA and MyD88 mRNA in I group were higher than those in S group at each time point after reperfusion ($p<0.05$) (Fig. 3B, 4B). The results suggested that TLR4 in right hippocampus CA1 region was activated by ischemia-reperfusion and blocked by anti-TLR4. The downregulation of MyD88 mRNA by anti-TLR4 suggests that MyD88 might be involved in the TLR4-mediated signal pathway activated by ischemia-reperfusion.

**Activation of NF-κB**  When electrophoretic gel mobility shift assay (EMSA) was used to examine activation of NF-κB in right hippocampal CA1 region in each group at each time point after ischemia-reperfusion, no protein product of NF-κB was observed in S group. However, a significant activation of NF-κB in I group was detected. In I group, the band of NF-κB became detectable at 12 h, reached the maximal level at 48 h after reperfusion, then decreased gradually at 72 h. Nevertheless, activation of NF-κB in T group at each time point after ischemia-reperfusion was lower than that in I group ($p<0.05$; Fig. 5). In the TLR4-mediated MyD88-dependent signaling pathway, NF-κB has been previously reported activated by MyD88. Comparison of these results to those in Fig. 5 revealed that activation of NF-κB in I group induced by MyD88 was first detected at 12 h and reached maximum at 48 h, indicating clearly the nuclear translocation of NF-κB. The present results suggest that ischemia-reperfusion activated the expression of NF-κB; NF-κB might be involved in the TLR4-mediated signal pathway activated by ischemia-reperfusion.

**Immunohistochemistry Staining of NF-κB**  To explore the nuclear translocation of NF-κB, we examined the expression of NF-κB by immunohistochemistry staining in each group at each time point after ischemia-reperfusion. The positive products for NF-κB showed brown, and mainly expressed in cytoplasm in S group, while mainly expressed in nucleus in I group. Positive products for NF-κB were expressed in cytoplasm and nucleus in T group (Fig. 6).

**Expression of IL-1β Protein Levels**  Nuclear translocation of NF-κB has been reported to induce expression of in-

![Fig. 5. Identification of Activated NF-κB in DNA–Protein Complex by EMSA](image)

Equal amount samples of nuclear extracts (7 μg) from right hippocampus in each group at each time point were incubated with binding reaction. The DNA–protein complexes and unbound probe (free probe) were separated electrophoretically on 6.5% polyacrylamide gel. NS indicates the nonspecific DNA-binding band (A). The IDV of EMSA in each group (B). Data are expressed as mean±S.D. from six animals. * $p<0.05$ compared with S group or T group.

![Fig. 6. Sections of 4% Paraformaldehyde-Fixed, Paraffin-Embedded Right Hippocampal Formation Were Immunohistochemically Stained to Reveal CA1 Region](image)

Positive products for NF-κB showed brown. A representative histology section 48 h after ischemia-reperfusion in I group in nucleus and in cytoplasm in S group is shown with positive products for NF-κB expressed in cytoplasm and nucleus in T group ($n=6$). Bar=25 μm.

![Fig. 7. Expression of IL-1β Protein in Each Group in Mice Right Hippocampal CA1 Region by Western Blot at Ischemia-Reperfusion 12, 24, 48 and 72 h (A) and the IDV of IL-1β in Each Group with Different Reperfusion Time Point (B)](image)

Data were expressed as mean±S.D. from six independent animals ($n=6$). * $p<0.05$ compared with S group or T group.
flammasory factors. After nuclear translocation of NF-kB was detected, to investigate whether inflammatory factors were induced, we then determined the IL-1β protein levels by Western blot in each group at each time point. The results from Western blot analysis of IL-1β showed that they were found at 12 h, reaching peak at 48 h after reperfusion, then decreasing gradually in I group (Fig. 7A), which was coincident with the expression of NF-xB, TLR4 mRNA, MyD88 mRNA and NF-xB. Furthermore, the IL-1β protein levels in T group and S group at each time point after ischemia-reperfusion were lower than those in I group (p<0.05; Fig. 7B).

DISCUSSION

Cerebral ischemia-reperfusion injury is a complex pathophysiologic process including a cascade reaction such as releasing of inflammatory cytokines. TLR4 is a type I transmembrane protein that contains a large, leucine-rich repeat in the extracellular region and a Toll/IL-1 receptor homology (TIR) domain in the cytoplasmic region. TLR4 is a key component of the innate immune system, functioning as a pattern recognition receptor that recognizes lipopolysaccharide (LPS), one of the most immunostimulatory glycolipids constituting the outer membrane of Gram-negative bacteria. After being stimulated by some microbial agents, TLR4-mediated MyD88-dependent signaling pathway triggers a cascade of cellular signals that leads to inflammatory cytokines expression such as IL-1β. There is increasing evidence supporting the notion that a link exists between innate immune/inflammatory responses and ischemia-induced neuronal damage. Recent studies have reported that TLR4-mediated NF-xB signaling contributes to myocardial ischemia reperfusion injury and TLR4 deficiency protects the myocardium from ischemic injury. TLR4 is also involved in the pathogenesis of I/R injury in liver, kidney and lung tissues. Some studies have shown that cerebral ischemia-reperfusion injury is reduced in TLR4-deficient mice and activation of TLR4 signaling contributes to hippocampus neuronal death following global cerebral ischemia/reperfusion. However, whether TLR4-mediated MyD88-dependent signaling pathway is activated after ischemia-reperfusion has not been formally demonstrated.

In the present study, TLR4 signaling pathway was inhibited by specific TLR4 antibodies (anti-TLR4) binding protein to prevent TLR4 from interacting with its receptors. Mice cerebral ischemia was induced by occlusion of CCA bilaterally. The mice in T group and I group were induced through 12 min of ischemia and sacrificed at different reperfusion time points. We selected the CA1 area of hippocampus of 12 min of ischemia and sacrificed at different reperfusion time points. We selected the CA1 area of hippocampus of cerebral ischemia-reperfusion has not been formally demonstrated. MyD88 recruitment to the TIR domain of TLR4 results in activation of NF-xB which produces proinflammatory cytokines such as interleukin-1β (IL-1β). IL-1β, a representative inflammatory cytokine, has been reported to play an important role in cerebral ischemia-reperfusion injury. In the present study, we found that activation of NF-xB in I group induced by TLR4-MyD88 was first detected at 12 h and maximally at 48 h, clearly indicating the nuclear translocation of NF-xB. However, TLR4 receptors blocked by anti-TLR4 resulted in down-regulation of NF-xB, IL-1β which suggest that TLR4-mediated MyD88-dependent signaling pathway activated after ischemia-reperfusion may be involved in the mechanism of ischemia-reperfusion through upregulation of NF-xB, IL-1β. Our study preliminarily suggests that TLR4 blocking method may be applicable for the treatment of cerebral ischemia-reperfusion injury.

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REFERENCES