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Lipopolysaccharide Preconditioning Induces Neuroprotection Against Early Brain Injury After Experimental Subarachnoid Hemorrhage

Lipopolisakkarit Ön Koşullandırması Deneysel Subaraknoid Kanama Sonrasında Erken Beyin Hasarına Karşı Nörolojik Korumayı İndükler

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ABSTRACT

AIM: Subarachnoid hemorrhage (SAH) is a devastating neurological injury associated with significant morbidity and mortality. We postulated that lipopolysaccharide (LPS) preconditioning induces neuroprotection against early brain injury (EBI) after experimental SAH.

MATERIAL and METHODS: 72 male Sprague-Dawley rats (250 to 300 g) were used. SAH was produced by injecting autologous arterial blood into the pre-chiasmatic cistern. Rats were given an intraperitoneal injection of LPS 24 hours prior SAH. Matrix metalloproteinase 9 (MMP-9) protein expression was measured by western blot; apoptosis in the cerebral cortex were studied by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) and 4'6-diamidino-2-phenylindole dihydrochloride (DAPI) staining at 24 h after SAH. Brain water content was also examined.

RESULTS: MMP-9 expression was increased after SAH and decreased by LPS preconditioning at 24 h after SAH. The number of neuronal death in cortex was increased after SAH and decreased by LPS preconditioning. In addition, brain water content was attenuated by LPS preconditioning.

CONCLUSION: LPS preconditioning could modulate MMP-9 and therefore induce neuroprotection against EBI after experimental SAH.

KEYWORDS: Cell death, Early brain injury, Matrix metalloproteinase 9, Subarachnoid hemorrhage, Preconditioning

ÖZ

AMAÇ: Subaraknoid kanama (SAK) önemli morbidite ve mortaliteyle ilişkili, çok ciddi bir nörolojik hasardır. Deneysel SAK sonrasında erken beyin yaralanmasına karşı lipopolisakkarit (LPS) ön koşullandırmasının nörolojik korumayı indüklediğini düşündük.

YÖNTEM ve GEREÇLER: 72 erkek Sprague-Dawley sıçanı (250 - 300 g) kullanıldı. Prekiazmatik sisterne otolog arteriyel kan enjeksiyonuyla SAK oluşturuldu. SAK oluşturulmasından 24 saat önce sıçanlara intraperitoneal LPS enjeksiyonu yapıldı. Matriks metalloproteinaz 9 (MMP-9) protein ifadesi western blot ile ölçüldü; serebral kortekste apoptoz SAK'den 24 saat sonra terminal deoksinükleotidil transferaz aracılıklı deoksiüridin trifosfat biotin çentikli uç etiketleme (TUNEL) ve 4'6-diamidino-2-fenilindol dihidroklorür (DAPI) boyamasıyla çalışıldı. Beyin su içeriği de incelendi.

BULGULAR: MMP-9 ifadesi SAK sonrasında artmış ama SAK'dan 24 saat sonra LPS ön koşullandırması yapılmasıyla azalmıştı. Kortekste nöronal ölüm sayısı SAK'dan sonra artmış ve LPS ön koşullandırmasıyla azalmıştı. Ayrıca beyin su içeriği LPS ön koşullandırması ile azalmıştı.

SONUÇ: LPS ön koşullandırması MMP-9 modülasyonu yapabilir ve böylece deneysel SAK sonrasında erken beyin hasarına karşı nörolojik korumayı indükleyebilir.

ANAHTAR SÖZCÜKLER: Hücre ölümü, Erken beyin hasarı, Matriks metalloproteinaz 9, Subaraknoid kanama, Ön koşullandırma

INTRODUCTION

Subarachnoid hemorrhage (SAH) is associated with high morbidity and mortality worldwide. Early brain injury (EBI) is the most common cause of morbidity and mortality in SAH patients (2,9). Treatment of EBI is considered a major goal in the management of patients surviving SAH. The term EBI refers to immediate injury to the brain as a whole, within the first 48 h of the ictus, secondary to SAH (12). Neuronal cell death results in cytotoxic edema, which contributes to the poor outcomes of SAH (19).

Lipopolysaccharide (LPS), known as a surface component of gram-negative bacteria, modulates the immune system through activation of Toll-like receptor 4 (TLR4). Previous studies suggested that low-dose exposure to LPS-induced cross-tolerance wherein protection occurred against heterologous injury unrelated to LPS, such as ischemia (16,17). Be-

sides, another study revealed that High-Mobility Group Box 1 (HMGB1) triggered matrix metalloproteinase 9 (MMP-9) up-regulation in neurons and astrocytes predominantly via TLR4 after cerebral ischemia (15). Recently, MMP-9, which belongs to a large family of endopeptidases, has been suggested to contribute to neuronal apoptosis during EBI after SAH (4,5,21).

Therefore, in this study, we employed LPS to investigate whether LPS preconditioning could induce neuroprotection against EBI after experimental SAH.

MATERIAL and METHODS

Animals

Seventy-two male Sprague-Dawley rats (250 to 300 g) purchased from the Experimental Animal Center of Sichuan University were randomly assigned to four groups with 18 animals in each group: sham operated, SAH, SAH with LPS preconditioning, and SAH treated with vehicle (saline preconditioning) groups. The rats were housed in temperature- and humidity-controlled animal quarters with a 12h light/dark cycle. All the animal procedures were evaluated and approved by the Animal Care and Use Committee at Sichuan University in Chengdu, China.

LPS Preconditioning and SAH Model

Rats were given an intraperitoneal injection of LPS (200 mg/kg; Escherichia coli serotype 055:B5; Sigma) 24 hours prior to surgery. This dosage was chosen based on previous studies of LPS-induced fever and systemic inflammation (6). Pilot experiments with control rats given higher dose of LPS showed unacceptable mortality within 24 h. For surgery, SAH induction was performed as reported previously with slight modifications (14). Briefly, male S-D rats were anesthetized with chloral hydrate (40 mg/kg, intraperitoneal injection); animal head was fixed in the stereotactic frame. A needle with a rounded tip and a side hole was tilted 30 degrees anteriorly and placed 7.5 mm anterior to bregma in the midline, with the hole facing the right side. The needle was lowered until the tip reached the base of the skull, 2–3 mm anterior to the chiasma (approximately 10 mm from the brain surface), and retracted 0.5 mm. The hole was plugged with bone wax before insertion of the needle. 250 µl blood was withdrawn from the femoral artery and injected intracranially through the needle at a pressure equal to the mean arterial blood pressure (80–100 mmHg). Finally, the needle was removed and incisions closed. In sham group, rats were treated by the same protocol as described above, except that no blood was injected after the needle was introduced. In vehicle group, rats were treated with the same volume of vehicle (saline instead of LPS) 24 hours before surgery.

Brain Water Content

Rat brains were removed at 24 h (n = 6 in each group) after SAH. Immediately, the entire brain was weighed after removal (wet weight) and then weighed again after drying in an oven at 105°C for 24 hours (dry weight), as described previously(10). The percentage of water content was calculated as [(wet weight - dry weight)/wet weight] x100%.

TUNEL and DAPI Staining to Evaluate Apoptosis

At 24 h after SAH, rats were deeply anesthetized and intracardially perfused with ice-cold PBS, pH 7.4, followed by 4% paraformaldehyde in PBS, pH 7.4 (n =6 in each group). The brains were removed, immersed in 4% paraformaldehyde/PBS overnight at 4°C. Coronal sections (5 µm thick) were prepared using a microtome. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) assays for apoptosis were performed according to the manufacturer's instructions (In Situ Cell Death Detection Kit, TMR red, Roche). For staining total nuclei and monitoring, intact, condensed, and fragmented nuclei, TUNEL-stained cells were counterstained with 4'6-diamidino-2-phenylindole dihydrochloride (DAPI, H-1200, VECTOR).

Preparation of Tissue Extracts

At 24 h after SAH, rats (n=6 in each group) were deeply anesthetized and then the brains were removed quickly and cerebral cortexes were dissected and frozen immediately in liquid nitrogen, and stored at -80°C. Sham operated control rats were killed at the same time. Brain tissue extracts were prepared as previously described (7). Briefly, brain samples were homogenized in lysis buffer on ice. After centrifugation, supernatant was collected, and total protein concentrations were determined using the Coomassie Brilliant Blue Method.

Western Blot

Western blot analysis was performed as described previously (20). Briefly, equal amounts of protein were loaded in each lane of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), electrophoresed and transferred to a nitrocellulose membrane. The membrane was blocked with MMP-9 mouse monoclonal antibody (Santa Cruz) and probed with anti-mouse IgG-horseradish peroxidase conjugated antibody. Densitometry analysis was performed with the ChemiDoc detection system (Bio-Rad) and Quantity One software (Bio-Rad).

Statistics

Data were expressed as mean±SD. Statistical differences between individual groups were analysed using one-way ANOVA. P value of <0.05 was considered statistically significant.

RESULTS

Brain Water Content

Brain water content was significant increased in rats at 24 hours after SAH when compared with sham operated group ($p<0.001$). LPS preconditioning decreased water content to a level similar to that of sham operated group ($p<0.001$ vs SAH group; $p>0.05$ vs sham operated group) (Figure 1).

TUNEL and DAPI

Apoptotic neurons with TUNEL and DAPI staining were observed in cortices of rats. The number of neuronal death increased significantly compared with sham operated

group at 24 hours after SAH (Figure 2). LPS preconditioning significantly attenuated the number of neuronal death compared with vehicle group (Figure 2).

Western Blot of MMP-9 Expression

Protein level of MMP-9 in the rat cerebral cortex was evaluated using western blot. At 24 hours after SAH, significantly increased expression of MMP-9 was found in cortex of SAH group compared with sham operated group ($p<0.001$, Figure 3). The densitometric analysis showed that LPS preconditioning significantly reduced the protein expression of MMP-9 compared with vehicle group ($p<0.001$, Figure 3).

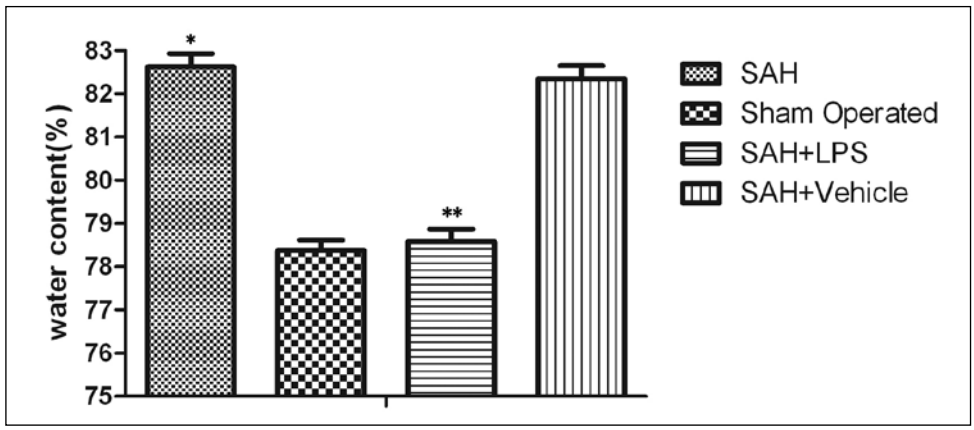

Figure 1: Alterations in brain water content in each SAH group (n=6). Brain water content was significant increased after SAH compared with sham operated group (*$p<0.001$). LPS preconditioning decreased water content to a level similar to that of sham operated group (**$p<0.001$ vs SAH group; $p>0.05$ vs sham operated group).

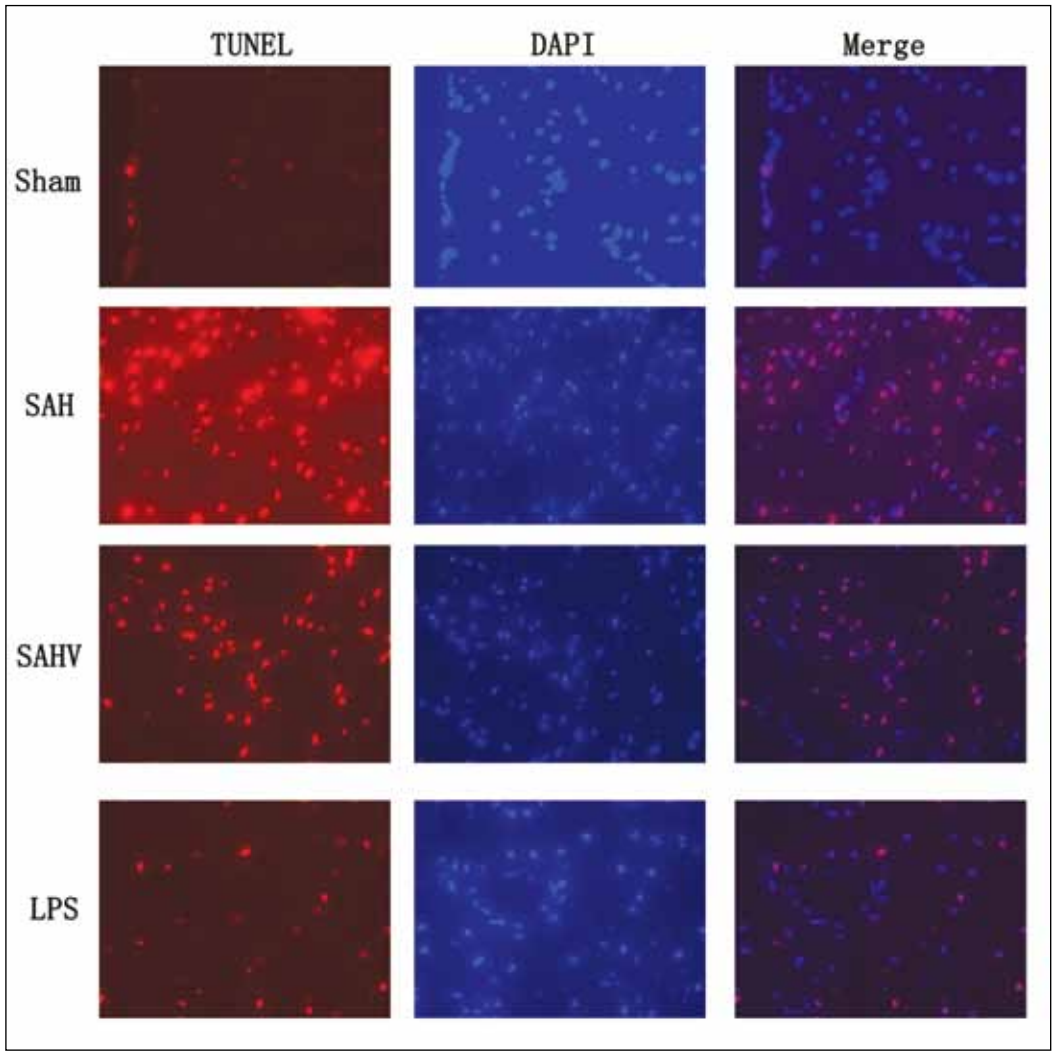

Figure 2: Detection of apoptosis in cortices of rats by TUNEL and DAPI staining. The number of neuronal death increased significantly compared with the sham operated group after SAH. LPS preconditioning significantly attenuated the number of neuronal death compared with vehicle group.

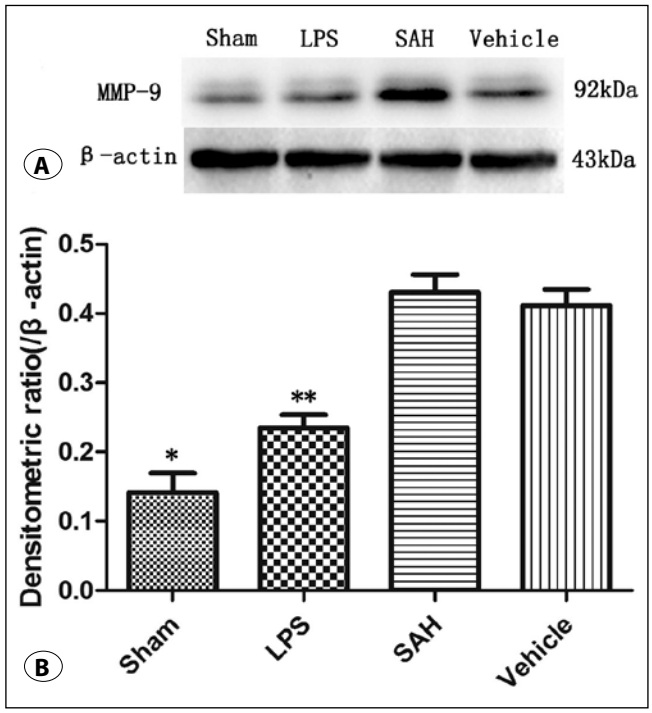

Figure 3: A) Representative bands for MMP-9 protein are shown at 24 h after SAH. **B)** MMP-9 expression was reduced significantly by LPS preconditioning (**p < 0.001 vs. SAH, b), but was still higher than that of the sham group (*p < 0.001 vs. sham, b).

DISCUSSION

Subarachnoid hemorrhage is one of the most serious cerebrovascular conditions; it has a mortality rate of almost 45%, and 30% of survivors experience moderate to severe disability (1). Although there have been some advances in treatment for SAH, the morbidity and mortality rates have not changed in recent years (11,18). More and more researchers have suggested that EBI is the primary cause of mortality in SAH patients (2). Apoptosis is involved in the pathological process of EBI. Neuronal apoptosis contributes to cytotoxic edema, which usually develops early after brain injury (13).

In the present study, we found that MMP-9 was activated in the early phase after SAH in rats and LPS preconditioning significantly reduced the protein expression of MMP-9. Besides, we observed significantly increased neuronal death in cortices of rats at 24h after SAH and LPS preconditioning significantly attenuated the number of neuronal death. After LPS preconditioning, we could induce neuroprotection against EBI after experimental SAH. Although the mechanisms that underlie the various forms of preconditioning are not well understood, they share a common link: small doses of an otherwise harmful stimulus induce protection against subsequent injurious challenge. It is known that LPS modulates the immune system through activation of TLR4 and low-dose exposure to LPS induced cross-tolerance wherein protection occurred against heterologous injury unrelated to LPS. Besides, a previous study found a new TLR4/MMP-9 signaling pathway (15). MMP-9 belongs to a large family of endopeptidases that are able to cleave extracellular matrix proteins. MMP-9 has been implicated in the pathogenesis of brain injury after ischemia and a number of neurodegenerative disorders (8,22). Activation of MMP-9 degrades components of extracellular matrix and results in the disruption of blood-brain barrier and cell death, both contributing to the formation of brain edema, either vasogenic or cytotoxic (2). Recently, several researches suggested it that MMP-9 contributed to neuronal apoptosis during EBI after SAH. Another previous study reported that MMP-9 contributed to the development of EBI after SAH by promoting cerebral edema formation (3).

In summary, this study demonstrated that MMP-9 contributed to neuronal apoptosis during EBI after SAH. LPS preconditioning could modulate MMP-9 through the activation of TLR4 and therefore induce neuroprotection against EBI after experimental SAH.

REFERENCES

1. Brisman JL, Song JK, Newell DW: Cerebral aneurysms. N Engl J Med 355:928-939, 2006
2. Cahill J, Calvert JW, Zhang JH: Mechanisms of early brain injury after subarachnoid hemorrhage. J Cereb Blood Flow Metab 26:1341-1353, 2006
3. Feiler S, Plesnila N, Thal SC, Zausinger S, Scholler K: Contribution of matrix metalloproteinase-9 to cerebral edema and functional outcome following experimental subarachnoid hemorrhage. Cerebrovasc Dis 32:289-295, 2011
4. Guo Z, Sun X, He Z, Jiang Y, Zhang X: Role of matrix metalloproteinase-9 in apoptosis of hippocampal neurons in rats during early brain injury after subarachnoid hemorrhage. Neurol Sci 31:143-149, 2010
5. Guo Z, Sun X, He Z, Jiang Y, Zhang X, Zhang JH: Matrix metalloproteinase-9 potentiates early brain injury after subarachnoid hemorrhage. Neurol Res 32:715-720, 2010
6. Hang CH, Shi JX, Tian J, Li JS, Wu W, Yin HX: Effect of systemic LPS injection on cortical NF-kappaB activity and inflammatory response following traumatic brain injury in rats. Brain Res 1026:23-32, 2004
7. Kawakita K, Kawai N, Kuroda Y, Yasashita S, Nagao S: Expression of matrix metalloproteinase-9 in thrombin-induced brain edema formation in rats. J Stroke Cerebrovasc Dis 15:88-95, 2006
8. Kiaei M, Kipiani K, Calingasan NY, Wille E, Chen J, Heissig B, et al: Matrix metalloproteinase-9 regulates TNF-alpha and FasL expression in neuronal, glial cells and its absence extends life in a transgenic mouse model of amyotrophic lateral sclerosis. Exp Neurol 205:74-81, 2007
9. Kusaka G, Ishikawa M, Nanda A, Granger DN, Zhang JH: Signaling pathways for early brain injury after subarachnoid hemorrhage. J Cereb Blood Flow Metab 24:916-925, 2004
10. Lundblad C, Bentzer P: Effects of L-arginine on cerebral blood flow, microvascular permeability, number of perfused capillaries, and brain water content in the traumatized mouse brain. Microvasc Res 74:1-8, 2007

11. Muroi C, Frei K, El Beltagy M, Cesnulis E, Yonekawa Y, Keller E: Combined therapeutic hypothermia and barbiturate coma reduces interleukin-6 in the cerebrospinal fluid after aneurysmal subarachnoid hemorrhage. J Neurosurg Anesthesiol 20:193-198, 2008
12. Ostrowski RP, Colohan AR, Zhang JH: Molecular mechanisms of early brain injury after subarachnoid hemorrhage. Neurol Res 28:399-414, 2006
13. Park S, Yamaguchi M, Zhou C, Calvert JW, Tang J, Zhang JH: Neurovascular protection reduces early brain injury after subarachnoid hemorrhage. Stroke 35:2412-2417, 2004
14. Prunell GF, Mathiesen T, Diemer NH, Svendgaard NA: Experimental subarachnoid hemorrhage: Subarachnoid blood volume, mortality rate, neuronal death, cerebral blood flow, and perfusion pressure in three different rat models. Neurosurgery 52:165-175; discussion 175-166, 2003
15. Qiu J, Xu J, Zheng Y, Wei Y, Zhu X, Lo EH, et al: High-mobility group box 1 promotes metalloproteinase-9 upregulation through Toll-like receptor 4 after cerebral ischemia. Stroke 41:2077-2082, 2010
16. Rosenzweig HL, Lessov NS, Henshall DC, Minami M, Simon RP, Stenzel-Poore MP: Endotoxin preconditioning prevents cellular inflammatory response during ischemic neuroprotection in mice. Stroke 35:2576-2581, 2004
17. Rosenzweig HL, Minami M, Lessov NS, Coste SC, Stevens SL, Henshall DC, et al: Endotoxin preconditioning protects against the cytotoxic effects of TNF alpha after stroke: A novel role for TNF alpha in LPS-ischemic tolerance. J Cereb Blood Flow Metab 27:1663-1674, 2007
18. Schievink WI, Riedinger M, Jhutty TK, Simon P: Racial disparities in subarachnoid hemorrhage mortality: Los Angeles County, California, 1985-1998. Neuroepidemiology 23:299-305, 2004
19. Schubert GA, Poli S, Schilling L, Heiland S, Thome C: Hypothermia reduces cytotoxic edema and metabolic alterations during the acute phase of massive SAH: A diffusion-weighted imaging and spectroscopy study in rats. J Neurotrauma 25:841-852, 2008
20. Sifringer M, Stefovska V, Zentner I, Hansen B, Stepulak A, Knaute C, et al: The role of matrix metalloproteinases in infant traumatic brain injury. Neurobiol Dis 25:526-535, 2007
21. Wang Z, Meng CJ, Shen XM, Shu Z, Ma C, Zhu GQ, et al: Potential contribution of hypoxia-inducible factor-1alpha, aquaporin-4, and matrix metalloproteinase-9 to blood-brain barrier disruption and brain edema after experimental subarachnoid hemorrhage. J Mol Neurosci 48:273-280, 2012
22. Zhao BQ, Tejima E, Lo EH: Neurovascular proteases in brain injury, hemorrhage and remodeling after stroke. Stroke 38:748-752, 2007