Postprint: Application of Lipopolysaccharide in Traumatic Brain Injury Inflammatory Response

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Abstract

Objective: To evaluate and analyze the application value of lipopolysaccharide in the inflammatory response of traumatic brain injury (TBI). Methods: A total of 86 TBI patients admitted to the Department of Neurology of Foshan Nanhai Third People' s Hospital and the Department of Neurosurgery of Zhujiang Hospital of Southern Medical University from June 2014 to December 2016 were selected and divided into three groups according to Glasgow Coma Scale score: mild group (33 cases), moderate group (24 cases), and severe group (29 cases). Additionally, 28 healthy individuals who underwent physical examination at our hospital during the same period were selected as the control group. Serum levels of TNF- , IL-6, and IL-1 were detected by ELISA, and nitric oxide content was measured by the Griess method. Results: The serum TNF- concentrations in the mild, moderate, and severe groups were all elevated at admission, with the highest level on day 3, gradually decreasing on days 5 and 7, but still higher than those in the control group. The serum IL-6 concentrations in the mild, moderate, and severe groups were all elevated at admission, with the highest level on day 5, beginning to decrease on day 7, but still higher than those in the control group; TBI severity grading was correlated with serum IL-6 concentration ($r=0.925$, $P<0.05$). The serum IL-1 concentrations in the mild, moderate, and severe groups were all elevated at admission, increasing slowly on days 1, 3, and 5, with a more significant increase on day 7; the increase in the severe group was significantly higher than that in the control, mild, and moderate groups ($P<0.05$). TBI severity grading was correlated with serum IL-1 concentration ($r=1.267$, $P<0.05$). The serum nitric oxide concentrations in the mild, moderate, and severe groups were elevated at admission, with a more pronounced increase on day 3, gradually decreasing on days 5 and 7, but still higher than those in the control group; TBI severity grading was correlated with serum nitric oxide concentration ($r=0.847$, $P<0.05$). Conclusion: Lipopolysaccharide exhibits varying degrees of inflammatory response in TBI patients; changes in

serum concentrations of TNF- , IL-6, IL-1 , and nitric oxide are closely related to the severity of TBI; if concentrations remain persistently high, this indicates severe brain tissue damage and poor prognosis.

Full Text

Preamble

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Application of Lipopolysaccharide in the Inflammatory Response of Traumatic Brain Injury

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Abstract

Objective: To evaluate the application value of lipopolysaccharide (LPS) in the inflammatory response following traumatic brain injury (TBI). **Methods:** A total of 86 TBI patients admitted to the Department of Neurology at Third People' s Hospital of Nanhai District and the Department of Neurosurgery at Zhujiang Hospital of Southern Medical University between June 2014 and December 2016 were enrolled. Patients were divided into three groups based on Glasgow Coma Scale (GCS) scores: mild group (n=33), moderate group (n=24), and severe group (n=29). Additionally, 28 healthy individuals undergoing physical examination at our hospital during the same period were selected as the control group. Serum levels of TNF- , IL-6, and IL-1 were measured by ELISA, and nitric oxide content was detected by the GREISS method. **Results:** Serum TNF- concentrations were elevated in all TBI groups at admission, peaked on day 3, and gradually decreased on days 5 and 7, but remained higher than the control group. Serum IL-6 concentrations were also elevated at admission, peaked on day 5, began to decline by day 7, and remained above control levels. TBI severity grading correlated positively with serum IL-6 concentration ($r=0.925$, $P<0.05$). Serum IL-1 concentrations were elevated at admission, increased slowly on days 1, 3, and 5, and rose more significantly on day 7, with the severe group showing markedly greater increases than the control, mild, and moderate groups ($P<0.05$). TBI severity grading correlated with serum IL-1 concentration ($r=1.267$, $P<0.05$). Serum nitric oxide concentrations were elevated at admission, increased most noticeably on day 3, gradually decreased on days 5 and 7, and remained higher than the control group. TBI severity grading

correlated with serum nitric oxide concentration (r=0.847, P<0.05). **Conclusion:** LPS induces varying degrees of inflammatory response in TBI patients. Changes in serum TNF- , IL-6, IL-1 , and nitric oxide concentrations are closely related to TBI severity. Persistently high levels of these mediators indicate severe brain tissue injury and poor prognosis.

Keywords: traumatic brain injury; lipopolysaccharide; serum TNF- ; IL-6; IL-1 ; nitric oxide

Introduction

Traumatic brain injury (TBI) severely impacts human quality of life and lifespan, with significantly increased morbidity, disability, and mortality rates, imposing a substantial burden on families. Selecting appropriate methods for early protection against secondary brain injury and promoting functional recovery is critically important. Inflammatory cytokines constitute an essential component of the pathophysiological process following TBI. While they can enhance host resistance and effectively promote tissue repair, excessive activation triggers a robust systemic inflammatory response that exacerbates secondary brain injury. Studies have shown that stellate ganglion block can reduce inflammatory responses in TBI patients by inhibiting lymphocyte NF- B and AP-1 activation. Various interventions including glucocorticoids, endotoxin antibodies, TNF antibodies, IL-1 receptor antagonists, IL-6 receptor antagonists, bradykinin antagonists, escin, melatonin, and hemodialysis have achieved some success in suppressing or clearing inflammatory mediators, though results remain unsatisfactory.

Lipopolysaccharide (LPS), composed of core polysaccharide, O-antigen chain, and lipid A, is also known as endotoxin and constitutes the main component of Gram-negative bacterial outer membranes. Even slight increases in intracellular ROS levels can stimulate the FOXOs transcription factor protein family, enhancing their transcriptional activity. Consequently, LPS serves as a primary trigger for inflammation and innate immune responses. Whether blocking LPS-mediated microglial activation can inhibit inflammatory cytokine secretion and reduce brain tissue damage requires further investigation, and LPS may represent a novel prognostic indicator for TBI. While domestic literature extensively reports changes in TNF- , IL-6, and IL-1 following TBI and their clinical significance, no studies have examined the changing patterns of nitric oxide (NO) across different TBI severity levels or its relationship with prognosis. This study evaluates the value of LPS in TBI inflammatory responses by analyzing serum TNF- , IL-6, IL-1 , and NO levels, aiming to provide objective, scientific guidance for improving clinical treatment and patient outcomes.

1.1 General Information

We selected 86 TBI patients admitted to our institutions between June 2014 and December 2016, all confirmed by CT or MRI scanning. The cohort included 66 males and 20 females, aged 18-78 years (mean 45.79±12.83 years).

Patients were stratified into three groups based on Glasgow Coma Scale (GCS) scores: mild group (n=33, GCS 13-15 points, 38.37%), comprising 26 males and 7 females aged 18-62 years (mean 43.54±10.27 years); moderate group (n=24, GCS 9-12 points, 27.91%), with 19 males and 5 females aged 20-71 years (mean 45.81±11.45 years); and severe group (n=29, GCS 3-8 points, 33.72%), including 21 males and 8 females aged 23-78 years (mean 46.78±13.09 years). All patients were admitted within 24 hours post-injury.

Exclusion criteria included: (1) severe cardiovascular or cerebrovascular organic disease or hepatic/renal insufficiency; (2) hemorrhagic shock; (3) pregnancy or lactation; (4) pre-existing hematological disorders or infections; and (5) psychiatric illness. Among the 86 TBI patients, 51 had cerebral contusions (59.30%), 15 had traumatic subarachnoid hemorrhage (17.44%), 10 had subdural hematoma (11.63%), 6 had cerebral contusion with epidural hematoma (6.98%), and 4 had intracerebral hematoma (4.65%). All 29 patients in the severe group underwent craniotomy for hematoma evacuation or decompressive craniectomy.

Twenty-eight healthy individuals undergoing physical examination at our hospital during the same period served as controls. Following the Declaration of Helsinki and relevant Chinese clinical trial regulations, and with approval from our institutional ethics committee, all participants or their families provided informed consent. No statistically significant differences existed in age, gender distribution, or other baseline characteristics among the four groups ($P>0.05$), ensuring comparability.

1.2 Methods

All patients underwent fasting elbow venous blood collection (5 mL) on days 1, 3, 5, and 7 after admission, while controls provided a single 5 mL sample during examination. Samples were placed in dry tubes and stored at 37°C in a constant temperature incubator (Suzhou Jiangdong Precision Instruments) until processing. After centrifugation at 3000 r/min for 15 minutes at room temperature using a tabletop centrifuge (Thermo, Germany), supernatants were removed and serum was stored at -20°C (Haier refrigerator) until analysis. LPS (Escherichia coli, serotype 026:B6, Sigma) was diluted to 1 mg/mL with sterile 0.9% sodium chloride and stored refrigerated until use.

Serum levels of tumor necrosis factor- (TNF-), interleukin-6 (IL-6), and interleukin-1 (IL-1) were measured by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assess inflammatory marker changes. Nitric oxide (NO) content was determined by the GREISS method. Assay kits were purchased from Raybiotech (USA), with all other reagents being analytically pure.

1.3 Statistical Analysis

Data were analyzed using SPSS 18.0 statistical software. Measurement data are expressed as mean±standard deviation. Comparisons between two groups were

performed using t-tests, while correlations among variables were analyzed using Pearson correlation and multiple stepwise regression. P<0.05 was considered statistically significant.

2.1 Serum TNF- Concentration Changes in TBI Patients

Serum TNF- concentrations were elevated in mild, moderate, and severe groups at admission, peaked on day 3, and gradually decreased on days 5 and 7 while remaining higher than the control group . Correlation analysis revealed that TBI severity grading correlated with serum TNF- concentration (r=1.873, P<0.05).

2.2 Serum IL-6 Concentration Changes in TBI Patients

Serum IL-6 concentrations were elevated in all TBI groups at admission, peaked on day 5, began to decline by day 7, and remained above control levels . Correlation analysis demonstrated that TBI severity grading correlated with serum IL-6 concentration (r=0.925, P<0.05).

2.3 Serum IL-1 Concentration Changes in TBI Patients

Serum IL-1 concentrations were elevated in all TBI groups at admission, increased slowly on days 1, 3, and 5, and rose more significantly on day 7. The severe group showed markedly greater increases than the control, mild, and moderate groups (P<0.05) . Correlation analysis indicated that TBI severity grading correlated with serum IL-1 concentration (r=1.267, P<0.05).

2.4 Serum NO Concentration Changes in TBI Patients

Serum NO concentrations were elevated in all TBI groups at admission, increased most noticeably on day 3, gradually decreased on days 5 and 7, and remained higher than the control group . Correlation analysis showed that TBI severity grading correlated with serum NO concentration (r=0.847, P<0.05).

Discussion

Secondary brain damage following TBI represents a crucial factor contributing to high mortality and disability rates. TBI triggers massive release of inflammatory cytokines, which are now recognized as essential components in pathophysiological processes including cerebral edema, ischemia, and increased intracranial pressure. LPS, the primary component of endotoxin, can induce widespread inflammatory reactions. The transient upregulation of IL-1 and TNF- during acute inflammatory responses suggests that cytokine production is tightly controlled, subsequently triggering neurotrophic factor generation that facilitates injured neuron recovery. However, the shift in cytokine expression from initial neuroprotective effects to exacerbation of neuronal injury demonstrates the detrimental aspects of sustained microglial activation. TBI-induced local tissue damage triggers inflammatory responses essential for clearing cellular debris.

TNF- exhibits toxic effects on human cortical neurons and neuron-like cells, and microglia can be activated by LPS to release TNF- . TNF- expression can induce neuropathological changes and neurodegeneration in transgenic mice.

Our findings demonstrate that serum TNF- concentrations were elevated in mild, moderate, and severe groups at admission, correlating with TBI severity grading. Particularly in early TBI stages, severe destruction of neural cells and local vascular tissues triggers various inflammatory responses. Interleukins, cytokines secreted by leukocytes that regulate cell growth and differentiation, generate large quantities of pro-inflammatory factors (IL-6, IL-1 , and TNF-) in blood. These mediators affect nearly all immune cells and endothelial cells, participating in inflammatory reactions and tissue injury. Microglia play important roles in various neurological diseases and participate in brain injury and repair processes, providing neuroprotection and rapidly responding to pathological stimuli in the brain while engaging in a series of immune responses. Astrocytes also respond to different forms of TBI through changes in gene expression, morphogenesis, and cell proliferation.

LPS is a potent inflammatory inducer that rapidly activates microglia, releasing numerous pro-inflammatory cytokines and active products that exert toxic effects on neurons and serve as primary triggers for inflammation and innate immune responses. LPS can significantly increase TNF- expression in BV-2 cells and TNF- concentration in their supernatant, while effectively altering BV-2 cell morphology by enlarging cell bodies and shortening processes. IL-6 can disrupt microcirculation, damage neural cells, worsen cerebral edema, and aggravate brain tissue injury by activating platelets and granulocytes. Our results show that serum IL-6 concentrations were elevated in all TBI groups at admission, with TBI severity grading correlating with serum IL-6 concentration. IL-1 is primarily expressed on phagocytic microglia and macrophages, regulating late inflammatory responses in cerebral ischemia. Our findings indicate that serum IL-1 concentrations were elevated in all TBI groups at admission, with the severe group showing significantly greater increases than other groups, and TBI severity grading correlated with serum IL-1 concentration. NO is chemically highly reactive and can profoundly alter cellular lipopolysaccharide and protein structures, causing imbalance in signal transduction mechanisms and inducing cytotoxicity. Our results demonstrate that serum NO concentrations were elevated in all TBI groups at admission, with TBI severity grading correlating with serum NO concentration.

In summary, LPS induces varying degrees of inflammatory response in TBI patients. The changing patterns of serum TNF- , IL-6, IL-1 , and NO levels across different TBI severities and time points suggest LPS involvement in TBI pathophysiology, showing positive correlation with injury severity. This study employed dynamic monitoring of serum TNF- , IL-6, IL-1 , and NO concentrations, providing more comprehensive reflection of brain injury than single-marker detection. Consequently, measuring changes in these serum concentrations can effectively provide objective evidence of brain tissue injury severity,

enable early prognosis assessment, and prove crucial for clinical judgment and treatment. However, the mechanisms underlying differential inflammatory responses to LPS in TBI, whether serum TNF- , IL-6, IL-1 , and NO fully represent brain injury pathology, and whether drugs or methods can reduce or block LPS-mediated inflammatory cell activation to minimize secondary brain damage require further investigation.

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Note: Figure translations are in progress. See original paper for figures.