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Effects of interleukin-6 and IL-6/AMPK signaling pathway on mitochondrial biogenesis and astrocytes viability under experimental septic condition

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ABSTRACT

Objective: Interleukin-6 (IL-6) is a neuromodulation factor with extensive and complex biological activities. IL-6 has been reported to activate AMPK, while AMPK regulates mitochondrial biogenesis and autophagy. The aim of this study was to investigate the role of IL-6 in mitochondrial biogenesis using astrocytes under experimental septic condition and examined how IL-6/AMPK signaling pathway affected this process.

Methods: The primary cultures of cerebral cortical astrocytes were randomly allocated into six groups: control group, LPS + IFN-γ group, IL-6 group (LPS + IFN-γ + IL-6), C group (LPS + IFN-γ + IL-6 + Compound C), siRNA group (LPS + IFN-γ + IL-6 + IL-6R siRNA) and siRNA + C group (LPS + IFN-γ + IL-6 + IL-6R siRNA + Compound C). All groups were stimulated for 6 h. Cytokines and reactive oxygen species (ROS) analyses, detection of adenosine triphosphate (ATP), mtDNA content and cell viability, evaluation of the mitochondrial ultrastructure and volume density, western blots of proteins associated with mitochondrial biogenesis and phospho-adenosine monophosphate activated protein kinase (p-AMPK) were performed respectively.

Results: Compared with LPS + IFN-γ group, IL-6 group had milder ultrastructural damage of mitochondria, higher mtDNA content and mitochondrial volume density, higher expression of proteins associated with mitochondrial biogenesis (PGC-1α, NRF-1 and TFAM) and p-AMPK, and thus higher cell viability, whereas blocking IL-6/AMPK signaling pathway, the protective effect of IL-6 has been diminished, compared with IL-6 group.

Conclusion: IL-6 enhances mitochondrial biogenesis in astrocytes under experimental septic condition through IL-6/AMPK signaling pathway.

1. Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Sepsis-associated encephalopathy (SAE) is a diffuse brain dysfunction caused by sepsis without obvious central nervous system (CNS) infection, and it is the most common type of brain dysfunction in pediatric intensive care unit (PICU), with a morbidity rate of 9%–71% in sepsis [2]. Early diagnosis and treatment of SAE is of great significance in improving the prognosis and reducing the mortality rate of patients with sepsis.

Since mitochondrial dysfunction is common in sepsis and SAE, maintenance of mitochondrial homeostasis is crucial for cell survival and cellular energetic status. Mitochondrial biogenesis refers to the growth and division of existing mitochondria, which is a complex and multistep process [3]. A variety of nuclear and mitochondrial regulatory factors participate in this process, among which peroxisome proliferator activator receptor gamma coactivator-1 alpha (PGC-1α) is the major regulatory factor that regulates the expressions of nuclear respiratory factor-1(NRF-1)and mitochondrial transcription factor A (TFAM) [4–6]. NRF-1, a vital transcription factor, facilitates the transcription of many nuclear-encoded mitochondrial genes [7]. TFAM, a key activator essential for the replication, transcription, and stabilization of mtDNA [5,8].

Interleukin-6 (IL-6) is a neuromodulation factor with extensive and complex biological activities [9]. IL-6 transmits its signals through two distinct patterns termed classic signaling pathway and trans-signaling pathway. IL-6 plays an anti-inflammatory and regenerative role by forming IL-6/mIL-6R/gp130 complex in classical signaling pathway, while it acts as a proinflammatory cytokine by forming IL-6/sIL-6R/gp130 complex in trans-signaling pathway [10–12]. Recent studies have confirmed the divergent functions of IL-6. It is reported that IL-6 alleviates cerebral ischemic injury and plays a neuroprotective role in ischemic stroke by activating IL-6/IL-6R/STAT3 signaling pathway [13]. Moreover, IL-6 prevents cerebellar granule neurons (CGNs) from

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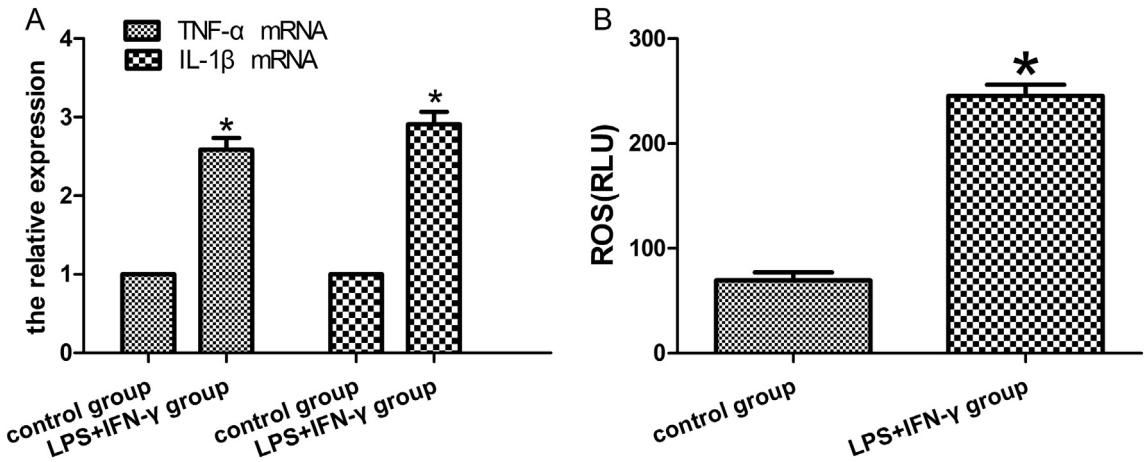

Fig. 1. Determination of biomarkers produced in astrocytes. A: Expressions of TNF-α mRNA and IL-1β mRNA. B: Level of ROS. Data are mean ± SD (n = 3–5 in each group), $^{*}P < 0.05$ versus control group.

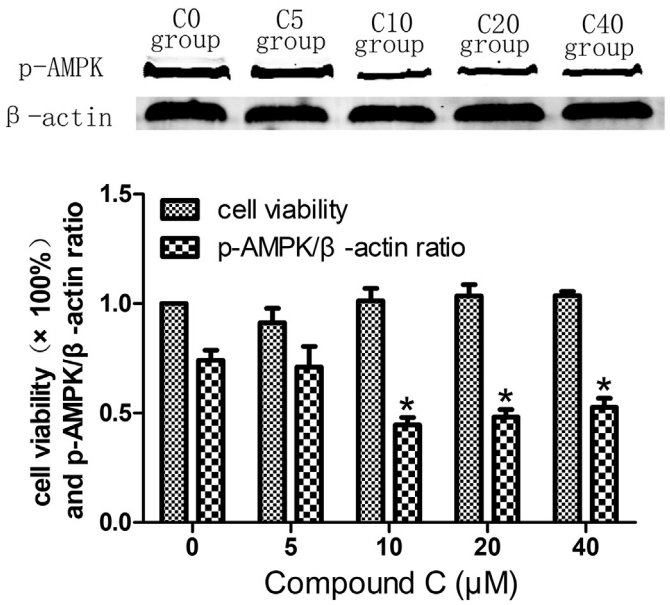

Fig. 2. Cell viability and inhibition of expression of p-AMPK in different concentrations of Compound C. Data are mean ± SD (n = 3 in each group), $^{*}P < 0.05$ versus 0 μM group.

NMDA-induced intracellular Ca^{2+} overload and cell death through gp130/JAK/STAT3, gp130/MAPK/ERK, and gp130/PI3K/AKT signaling pathways, demonstrating a neuroprotective effect of IL-6 [14]. On the contrary, the various detrimental effects of IL-6 in the central nervous system (CNS) appear to depend on trans-signaling pathway solely, by using sgp130 to block IL-6 trans-signaling specifically [15]. IL-6 is not the only mediator increased in inflammation and in sepsis. Many mediators exist in sepsis, inflammation, cancer and other diseases. Exposed to diverse stimuli in vitro, cultured epithelial cells can produce TNF-α and other cytokines like IL-1 and IL-6, involving in inflammation [16]. Moreover, the level of IL-6 elevated significantly, while the level of TNF-α significantly decreased in patients with bipolar disorder (BD), compared with healthy controls [17].

Adenosine monophosphate activated protein kinase (AMPK), which is highly conserved in all eukaryotes [18], is a master sensor of cellular energy status that regulates mitochondrial biogenesis, cell growth and proliferation, apoptosis, and autophagy [19,20]. AMPK is activated by the increase of adenosine monophosphate(AMP)/adenosine triphosphate(ATP)ratio and/or adenosine diphosphate (ADP)/ATP ratio under stress and oxidative stress conditions [21–24].

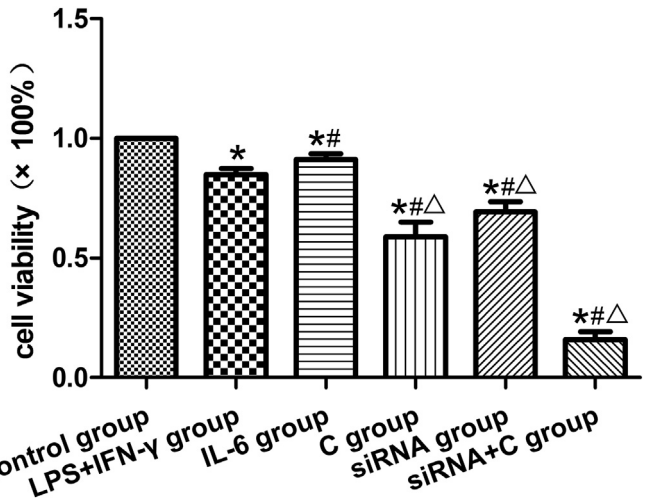

Fig. 3. IL-6 increases the cell viability under septic condition. Data are mean ± SD (n = 5 in each group), $^{*}P < 0.05$ versus control group, $^{\#}P < 0.05$ versus LPS + IFN-γ group, $^{\triangle}P < 0.05$ versus IL-6 group.

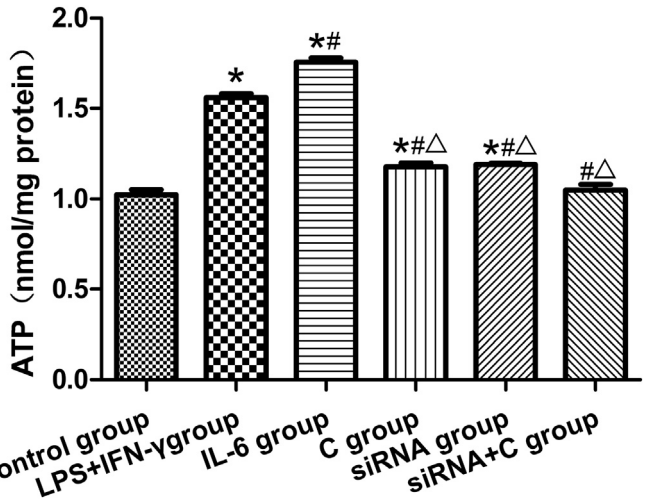

Fig. 4. IL-6 increases the level of ATP under septic condition. Data are mean ± SD (n = 3 in each group), $^{*}P < 0.05$ versus control group, $^{\#}P < 0.05$ versus LPS + IFN-γ group, $^{\triangle}P < 0.05$ versus IL-6 group.