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Research article

Acetyl-L-carnitineamelioratesmitochondrial damage and apoptosis following spinal cord injury in rats

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HIGHLIGHTS

- ALC prevented ultrastructural changes of mitochondria after SCI.
- ALC improved dynamic alternations of mitochondria after SCI.
- ALC suppressed cell apoptosis following SCI.

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ABSTRACT

Acetyl-L-carnitine (ALC) facilitates the entry and exit of fatty acids from mitochondria and plays an essential role in energy metabolism. Although ALC is known to exert neuroprotective effects in multiple neurological diseases, its effects on spinal cord injury (SCI)-induced mitochondrial impairments and apoptosis remain unclear. In this study, we aimed to evaluate the putative effects of ALC on mitochondrial dysfunction and apoptosis induced by SCI in a rodent model. Our results indicate that SCI elicits dynamic alternations in the expression of mitochondria-related proteins. Transmission electron microscopy analysis showed that ALC administration abrogated key ultrastructural abnormalities in mitochondria at 24 h after SCI by maintaining mitochondrial length, reducing the number of damaged mitochondria, and reversing mitochondrial score ($P < 0.05$ compared with SCI group). In addition, ALC administration maintained the mitochondrial membrane potential and mitochondrial Na^+-K^+-ATPase activity following SCI ($P < 0.05$ compared with SCI group). ALC administration reversed the downregulation of mitofusin 1 (Mfn1), Mfn2, Bcl-2, and the upregulation of dynamin-related protein 1 (Drp1), mitochondrial fission 1 (Fis1), Bcl-2-associated X protein (Bax) and cytosol cytochrome c (cyto-CytC) induced by SCI ($P < 0.05$ compared with SCI group). Finally ALC administration greatly reduced the percentage of apoptotic cells compared with the SCI group ($P < 0.01$). In conclusion, our findings demonstrated that ALC ameliorated SCI-induced mitochondrial structural alternations, mitochondrial dysfunction, and apoptosis.

1. Introduction

Spinal cord injury (SCI) is a severe neurological disorder that affects thousands of individuals annually and leads to lifelong disabilities in patients [1]. Despite the fact that the neuropathology of SCI remains unclear, mounting evidence suggests that apoptosis, the process of programmed cell death, in neurons and glia, contributes significantly to SCI-induced secondary damage [2–4].

Mitochondria are crucial intracellular organelles that determine cell fate via numerous essential biosynthetic and metabolic pathways [5]. Importantly, mitochondrial morphology and integrity play essential roles in eliciting cell apoptosis in response to various apoptotic signals [6]. During apoptosis, mitochondria undergo dynamic changes. The ultrastructure and morphological alternations in mitochondria are regulated by the equilibrium between mitochondrial fusion and fission events [7]. Several proteins have been implicated in the dynamic changes of mitochondria, including dynamin-related protein 1 (Drp1), mitochondrial fission 1(Fis1), mitofusin (Mfn) 1 and 2, cytochrome c (CytC), Bcl-2 and Bcl-2-associated X protein (Bax) [5].

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Acetyl-L-carnitine (ALC), a molecule generated by acetylation of carnitine in the mitochondria, has been shown to play an essential role in the maintenance of mitochondrial integrity and energy metabolism [8]. ALC has the ability to cross the blood-brain barrier [9]. Karalija et al. revealed that ALC administration rescued the death of motor neurons in adult rats following SCI [10]. In addition, intraperitoneal injection of 300 mg/kg ALC at 15 min, 30 min, 60 min, or 6 h post-injury greatly ameliorated mitochondrial dysfunction in a rat SCI model [11]. However, the molecular mechanism underlying the protective role of ALC in mitochondria remains unclear.

In the present study, we investigated the morphological alternations of mitochondria following SCI in rats, and determined the potential effects of ALC in terms of mitochondrial dysfunction. Moreover, putative molecular mechanisms underlying ALC-mediated mitochondrial protection were examined. Our findings may provide a novel strategy for preventing mitochondrial dysfunction following SCI.

2. Materials and methods

2.1. Preparation of mitochondrial, cytosol, and total protein extracts

Approximately 15 mm-length of spinal cord center at T10 was collected. The mitochondrial and cytosol proteins were extracted from tissues using protein extraction kit according to manufacture's instructions (Applygen Technologies Inc., Beijing, China). The protein concentration was determined using bicinchoninic acid (BCA) protein assay kit (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China). Mitochondria extracts were used for determination of mitochondrial function. Cytosol extracts were used for analyzing CytC expression. Total protein extracts were used for examining the protein expressions of Mfn1, Mfn2, Drp1, Fis1, Bcl-2 and Bax.

2.2. Transmission electron microscopy (TEM) analysis

Under anesthesia, rats were perfused with 0.25% glutaraldehyde and 4% paraformaldehyde (PFA) solution (1/1; v/v). Approximately 3-mm-length spinal cord tissue that was 1-mm away from the site of hit area (head-end or tail-end) was removed. Head-end spinal gray matter tissue (1-mm-length distal) was used for TEM analysis, while tail-end spinal cord tissue was fixed in 4% PFA followed by TUNEL analysis. For each sample, 10 serial ultrathin sections were cut into 70 nm thick. After an interval of 20 μm, another 10 serial sections (a package) were cut. A total of 100 sections were prepared, and one section was randomly selected from each package. Using this method, 10 sections were randomly selected and were double stained with uranyl acetate and lead citrate. Images were obtained under an H-7650 TEM (Hitachi Science Systems, Ltd, Japan) with a magnification of 20,000. Two or three different regions of interest (ROIs) per slice were captured. The number of ROIs was dependent on the size of neurons in each observation field, i.e., selecting 3 non-overlapping ROIs for big neurons, while 2 ROIs for small neurons. The cytosol of the cells was captured. The ultrastructure and function of mitochondria were analyzed as described previously [12,24,25]. The length of the maximum cross-sectional diameter was measured using the scale bar tool of Photoshop CS3 software (Adobe, USA). The degree of mitochondrial impairment was scored as follows: 0, normal mitochondrial; 1, normal ultrastructure of the crests and matrix but absence of granular deposits; 2, loss of matrix granules and clarification of the matrix without breaking of crests; 3, loss of matrix granules and uniform clarification of the matrix and disruption of crests; 4, loss of integrity of the mitochondrial membranes. The average mitochondrial length, score and the number of mitochondria per 1 μm^2 cytosolic area in each cell were calculated. All micrographs were examined in a blind fashion by two experts unknown about the animal study and sample source. Data were calculated and expressed as mean ± SD.

2.3. Evaluation of the mitochondrial membrane potential

The mitochondrial membrane potential was examined by a lipophilic cationic dye JC-1 [26]. A total of 100 μL mitochondrial extracts (1 μg/μL) were used for this analysis. JC-1 staining was conducted using JC-1 detection kit according to the manufacture's instructions (Beyotime Institute of Biotechnology, China). The fluorescence intensities were examined using a fluorescence spectrophotometer (Precision and Scientific Instrument Co., China). The ratio of the fluorescence intensity at 590 nm to the fluorescence intensity at 530 nm (F590 nm/F530 nm) was used as an indicator of the membrane potential.

2.4. Determination of mitochondrial Na^+-K^+-ATPase activity

A total of 400 μL mitochondrial extracts (1 μg/μL) were used for determination of mitochondrial Na^+-K^+-ATPase activity. The mitochondrial Na^+-K^+-ATPase was analyzed using a detection kit according to the manufacture's instructions (Nanjing Jiancheng Biology Engineering Institute, China). The absorbance was examined using a fluorescence spectrophotometer (Precision and Scientific Instrument Co., China). The mitochondrial Na^+-K^+-ATPase activity was assessed by the following equation: Mitochondrial Na^+-K^+-ATPase activity = $[(OD_{sample} - OD_{control})/OD_{standardsample}] \times$ the concentration of standard sample × dilution × 6/the concentration of sample.

3. Results

3.1. SCI altered the expression levels of mitochondria-related proteins

As shown by Fig. 1, the expression levels of Mfn1 and Mfn2 were significantly elevated at 4 h and 8 h after SCI ($P < 0.05$ compared with control), and were gradually decreased as time progressed (16 h and 24 h). In contrast, the protein levels of Drp1 and Fis1 were markedly downregulated at 4 h and 8 h after SCI ($P < 0.05$ compared with control), and were gradually elevated as time progressed (16 h and 24 h). In addition, cytosol CytC (cyto-CytC) was gradually upregulated following SCI in a time-dependent pattern ($P < 0.05$ compared with control). Based on these observations, we examined the morphological and dynamic changes of mitochondria at 24 h following SCI.

3.2. ALC reversed SCI-induced ultrastructural alternations in mitochondria

TEM analysis showed that SCI induced prominent morphological alternations in mitochondria at 24 h following injury (Fig. 2). The average cross-sectional length of mitochondria derived from the spinal cord of the SCI group was significantly decreased ($P < 0.01$ compared with the Sham operation group). Besides, the number of damaged mitochondria, the overall number of mitochondria, as well as the mitochondrial score were increased in the SCI group ($P < 0.01$ compared with Sham operation group). These data indicate that 24 h following SCI, mitochondria may indeed be undergoing fission leading to mitochondrial impairment. To determine the potential effects of ALC on mitochondrial impairment induced by SCI, a group of rats underwent ALC injection. We found that ALC injection efficiently maintained the length of mitochondria, reduced the number of damaged mitochondria, decreased the

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