Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

Mitochondrial fusion and fission after spinal sacord injury in rats

Yang Cao, Gang Lv, Yan-song Wang, Zhong-kai Fan*, Yun-long Bi, Liang Zhao, Zhan-peng Guo*

Department of Orthopaedics, The First Affiliated Hospital, Liaoning Medical University, 5-2 Renmin Street, Guta District, Jinzhou 121000, Liaoning Province, China

ARTICLE INFO

Article history:
Accepted 21 May 2013
Available online 28 May 2013

Keywords:
Mitochondria
Fusion
Fission
Spinal cord injury

ABSTRACT

Responsible for orchestrating cellular energy production, mitochondria are central to the maintenance of life and the gatekeepers of cell death. Its morphology is dynamic and controlled by continual and balanced fission and fusion events. In this study, we analyzed the mitochondrial dynamics and functions after spinal cord injury in rats and further to discuss the mechanisms of the mitochondria regulated cell injury during SCI. Using adult rat spinal cord injury model, it was found that the absolute number of mitochondria per area was significantly less and the individual mitochondrial cross-sectional area was significantly greater in the neurons of rats in SCI group than in the sham-operated group at 3 h and 6 h after SCI, and the reverse pattern at 12 h and 24 h after SCI. The results from Western blot and RT-PCR assays showed that the protein and mRNA levels of mitochondrial fusion-related genes (Mfn1 and Mfn2) decreased and fission-related genes (Drp1 and Fis1) increased at 3 h and 6 h after SCI. At 12 h and 24 h after SCI the reverse pattern of Mfn1, Mfn2, Drp1 and Fis1 expression was found. Taken together the results of the present study showed the mitochondrial tendency of elongation and fusion in the injured spinal cord at 3 h and 6 h after SCI, and the tendency of mitochondrial fission at 12 h and 24 h after SCI in our SCI models of rat. These findings have important implications for our understanding of the mechanisms of mitochondrial dynamics and functions after SCI injury. And mitochondrial fusion may potentially be used as a target for improving spinal cord function in the first 6 h after SCI. Mitochondrial fusion may be inhibited at 12–24 h after SCI for improving functional outcomes following SCI.

1. Introduction

Spinal cord injury (SCI) is a major public health problem, often leaving patients with lifelong disabilities. Neurological damage after acute SCI results from both primary mechanical injury and subsequent activation of cell death cascades mediating delayed tissue damage (Cai et al., 2012). Currently, there is no effective treatment, and the mechanisms underlying these neuropathological changes after SCI are not yet fully understood. It can be argued that therapeutic strategies targeting a specific biochemical cascade may not provide the best approach for promoting functional recovery. A "systems approach" at the sub-cellular level may provide a better strategy for promoting cell survival and function and, as a

*Corresponding authors. Fax: +86 416 4197639.
E-mail addresses: lvgang_ln@yeah.net (G. Lv), fanzk_ln@126.com (Z.-k. Fan).

0006-8993/$ - see front matter Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.05.033

consequence, improve functional outcomes following SCI. One such approach is to study the impact of SCI on the biology and function of mitochondria, which serve a major role in cellular bioenergetics, function, and survival. Mitochondria are ubiquitous intracellular organelles enclosed by a double membrane-bound structure. The primary function of mitochondria is the production of cellular energy in the form of adenosine triphosphate (ATP) by the mitochondrial respiratory chain through oxidative phosphorylation (McBride et al., 2006). Mitochondrial oxidative phosphorylation system consists of five multienzyme complexes (Complexes I–V) located in the mitochondrial membrane. Biochemical evidences suggested that the majority of cerebral ATP consumption is used for operation of the electrogenic activity of neurons (Chen et al., 2011). Adequate energy supply by mitochondria is essential for neuronal excitability and neuronal survival (Chuang, 2010). Mitochondrial impairment is hypothesized to be involved in cell injury during spinal cord injury. Assessment of mitochondrial bioenergetics is a valuable tool not only for studying the time course of mitochondrial change following SCI, but also for shedding light on the mechanisms responsible for the dysfunction of SCI. Mitochondria frequently change their shape by fusion and fission, and recent research on these morphological dynamics of mitochondria has highlighted their role in normal cell physiology and disease. The aim of the current study is to investigate the mitochondrial dynamics and functions after spinal cord injury in rats and further to discuss the mechanisms of the mitochondria regulated cell injury during SCI.

2. Results

2.1. *The effect of SCI on the mitochondrial morphology*

Changes in mitochondrial morphology in neurons from the injured spinal cord after SCI were shown in Fig. 1. In the typical micrograph sections of neurons, the numbers and the size of the mitochondria were confirmed per area by quantitative and statistical analyses. In quantitative analysis, random sections were studied, except that regions with swollen or less dense mitochondria were excluded to reduce bias. The results revealed that mitochondrial morphology changes during the first 24 h following SCI. The absolute number of mitochondria per area was significantly less and the individual mitochondrial cross-sectional area was significantly greater in the neurons of rats in SCI group at 3 h and 6 h after SCI than in the sham-operated group ($p<0.05$). At 12 h after SCI, the absolute number of mitochondria per area was significantly increased and the individual mitochondrial cross-sectional area was significantly decreased in the neurons. There was no significant difference about the mitochondrial data in the neurons of SCI group at 24 h after SCI and at 12 h after SCI.

2.2. *The SCI effect on the expression of mitochondrial fission proteins in the injured spinal cords*

Mitochondrial fission 1 (Fis1) and dynamin-related protein 1 (Drp1) are the main mitochondrial fission proteins in mammalian cells (Mai et al., 2010). Protein expression and mRNA of Drp1 and Fis1 were analyzed by western blot and Reverse Transcription-Polymerase Chain Reaction (RT-PCR). There was no significant difference in Drp1 and Fis1 in sham-operated group at different time after surgery at the spinal cord. As shown in Figs. 2 and 3, it revealed significant changes in the levels of mitochondrial fission proteins at 3 h, 6 h, 12 h and 24 h after SCI. The protein expression of Drp1 and Fis1 was significantly decreased in spinal cords extracts from SCI rats at 3 h and 6 h after SCI, then increased at 12 h after SCI and reached the apex at 24 h after SCI ($P<0.05$). Fis1 mRNA levels were dramatically reduced in the injured spinal cords at 3 h and 6 h after SCI, increased at 12 h and 24 h after SCI compared with sham-operated group. Measuring Drp1 mRNA by RT-PCR proved a little different. There was no significant difference about Drp1 mRNA in the neurons of SCI group and sham-operated group at 12 h, 24 h after SCI.

2.3. *The SCI effect on the expression of mitochondrial fusion proteins in the injured spinal cords*

In mammalian cells, the large mitochondrial GTPases Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) play important roles in mitochondrial fusion. As shown in Figs. 2 and 3, Western blot and RT-PCR results showed that Mfn1 and Mfn2 protein and mRNA were found in the cords of sham-operated group, and there was no significant difference in Mfn1 and Mfn2 protein and mRNA level in sham-operated group at different time after surgery at the spinal cord. In SCI group, the protein expression and mRNA of Mfn1 and Mfn2 increased in the injured spinal cords at 3 h and 6 h after SCI. Then they decreased at 12 h and 24 h after SCI.

3. Discussion

SCI causes marked neuropathological changes in the spinal cord, resulting in limited functional recovery. Currently, there is no effective treatment, and the mechanisms underlying these neuropathological changes are not clearly understood. Mitochondria are membrane-enclosed organelles which serve a wide variety of actions critical to cellular function, several of which are of particular importance to neuronal survival (Hoekstra et al., 2011). The primary function of mitochondria is to produce energy in the form of ATP via oxidative phosphorylation, in which electrons are transported down the electron transport chain (ETC) while generating a proton gradient. Moreover mitochondrial functions are greatly important for the nervous system, including protein importation, organellar dynamics, and programmed cell death (Uo et al., 2009). It is essential to maintain neuronal viability and neuronal function because of their ability to provide ATP, regulate calcium homeostasis and control Fe/S cluster biogenesis (DiMauro and Schon, 2008). Consistent with this critical role, mitochondrial impairment is hypothesized to contribute to cell injury during spinal cord injury (Lin and Beal, 2006). Mitochondrial fission and fusion play critical roles in maintaining functional mitochondria when cells experience metabolic or environmental stresses. It is described relatively recently and most extensively in yeast, occurs constantly and is thought to be critical for normal