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Effect of carnosine, methylprednisolone and their combined application on irisin levels in the plasma and brain of rats with acute spinal cord injury

Serdal Albayrak [a], İbrahim Burak Atci [a], Mehmet Kalayci [b], Musa Yilmaz [c], Tuncay Kuloglu [d], Suna Aydin [e], Mustafa Kom [f], Omer Ayden [a], Suleyman Aydin [c,*]

[a] Department of Neurosurgery, Elazig Education and Research Hospital, 23100, Elazig, Turkey
[b] Laboratory of Medical Biochemistry, Elazig Education and Research Hospital, Elazig 23100, Turkey
[c] Firat University, School of Medicine, Department of Medical Biochemistry (Firat Hormones Research Group), 23119 Elazig, Turkey
[d] Firat University, School of Medicine, Department of Histology&Embryology, 23119, Elazig, Turkey
[e] Cardiovascular Surgery- Anatomy, Elazig Education and Research Hospital, 23100, Elazig, Turkey
[f] Firat University, Veterinary of Medicine, Department of Surgery, Elazig 23119, Turkey

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SUMMARY

Spinal cord injury (SCI) might occur to anybody at any time and any age. In its treatment, methylprednisolone (MP) is a first choice worldwide, but there is still no significant breakthrough in truly beneficial treatment due to SCI's complex pathophysiology. We investigated the effect of carnosine, methylprednisolone (MP) and its combination on irisin levels in the plasma, brain and medulla spinalis tissues in SCI using a rat model. The rats were divided into 6 groups: I (Control, saline); II (sham animals with laminectomy without cross-clamping); III (SCI); IV (SCI treated with 150 mg/kg carnosine); V (SCI treated with 30 mg/kg methylprednisolone); and VI (SCI treated with a combination of carnosine and MP). The animals were given traumatic SCI after laminectomy, using 70-g closing force aneurysm clips (Yasargil FE 721). Irisin concentration was measured by ELISA. The distribution of irisin in brain and spinal cord tissues was examined by immunochemistry. Irisin was mainly expressed in the astrocytes and microglia of brain tissues, and multipolar neurones of the anterior horn of spinal cord tissue in rats of all groups, indicating that irisin is physiologically indispensable. MP and carnosine and the combination of the two, significantly increased irisin in plasma and were accompanied by a significant rise in irisin immunoreactivity of brain and spinal cord tissues of the injured rats compared with control and sham. This finding raises the possibility that methylprednisolone and carnosine regulate the brain and spinal cord tissues in SCI by inducing irisin expression, and may therefore offer a better neurological prognosis.

1. Introduction

Spinal cord injury (SCI) leads to a profound damage to the central nervous system and is associated with significant financial and personal burden to the patient at any age, but it generally occurs in young adults in the second and third decades of life (Kumamaru et al., 2012). Treatment is a challenging healthcare problem due to involvement of a complex cascade of secondary neurodegenerative events set in motion by the primary injury, coupled with the lack of available robust pharmacological and novel therapeutic approaches (Nasirinezhad et al., 2015). Glucocorticoid steroids, mainly methylprednisolone (MP), have been extensively used in the clinical treatment of spinal cord trauma beginning in the mid-1960s and throughout the 1970s. At a high dose level in the SCI rat model, there is a decrease in lactate accumulation (Farooque et al., 1996), inhibition of post-traumatic spinal cord ischemia (Holtz et al., 1990), attenuation of vascular permeability (Xu et al., 1992), decrease in inflammatory markers (Xu et al., 2001), improved neurological recovery, support for aerobic energy metabolism [improved adenosine triphosphate (ATP) and energy charge], and an anti-oxidant neuroprotective action (Behrmann et al., 1994; Holtz et al., 1990).

However, treatments remain limited, and are aimed mainly at supportive care due to the complex character of SCI (Nasirinezhad et al., 2015). Recent findings have indicated that SCI causes profound muscular atrophy, and bone loss with fragility. Moreover, other advances have identified a link between SCI and muscle metabolism, thereby identifying other tissues such as endocrine organs when irisin was firstly discovered in muscle tissues by Bostrom et al. (2012). They reported that irisin levels increased after exercise, helping convert white adipose to brown adipose tissue, thereby causing weight loss, identifying irisin as

* Corresponding author at: Firat University, School of Medicine, Department of Medical Biochemistry (Firat Hormones Research Group), 23119 Elazig, Turkey. Tel.: +90 5334934643; fax: +90 424 2379138.
E-mail address: saydin1@hotmail.com (S. Aydin).

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an anti-obesity hormone (Bostrom et al., 2012). Since increased irisin means more brown adipose tissue, this means that there is more capacity for releasing heat instead of using ATP synthesis (Bostrom et al., 2012). As mentioned before, MP supports aerobic energy metabolism through improved ATP and energy charge. Irisin is related with heat or ATP (Bostrom et al., 2012). Therefore there may be a link between the MP treatment of SCI, making investigation worthwhile into how irisin expression changes in SCI after treatment with MP. Irisin also regulates glucose homeostasis (Aydin, 2014; Bostrom et al., 2012). After the discovery of muscle-secreted irisin, the hormone was found to be almost ubiquitously present in biological tissues and its presence is not dependent on exercise, as it is present in the resting tissues (Aydin, 2014; Aydin et al., 2014b). Based on this knowledge, it was hypothesized that the muscle and other tissues, including brain tissue-secreted irisin, might have a link between endocrine functions (including irisin synthesis) of tissues and SCI. Since there is evidence that exercising paralyzed muscles can lead to a reversal of muscle atrophy over time after SCI (Taylor et al., 2011), exercise might prevent some of the early muscle changes after injury. A link probably exists between a reversal of muscle atrophy and irisin concentration, since it is known that irisin secretion from skeletal muscles increases after exercise (Aydin, 2014; Bostrom et al., 2012).

SCI may overwhelm cellular anti-oxidant defense and ultimately result in oxidative stress and secondary injury (Shaw et al., 1997). Like the anti-oxidant neuroprotective action of MP, carnosine (C) is also mainly found in muscle and brain tissues (Boldyrev, 2012; Boldyrev et al., 2013). This dipeptide (beta-alanyl-L-histidine) scavenges reactive oxygen species (ROS) to prevent peroxidation of cell membrane fatty acids during oxidative stress (Boldyrev, 2012; Coban et al., 2013). Thus, we hypothesized that carnosine might reverse the detrimental effects of SCI. However, its effects on irisin levels in the plasma, brain and medulla spinalis tissues have not been properly studied. Thus, we have investigated the effect of C, MP alone or in combination on irisin levels in these tissues of SCI rats.

2. Material and methods

The study was approved by the animal ethics committee of Firat University resolution (number 12) on 01.08. 2014, on the principles of the Guide to the Care and Use of Experimental Animals. 30 male adult Sprague–Dawley (SD) rats weighing 280–300 g were maintained under controlled environmental conditions in a 12/12-h light/dark cycle at 22 ± 1 °C, in a standard housing environment with food and water ad libitum. The animals were randomly divided into 6 experimental groups: Group I (Control, having nothing done, only saline); Group II (sham animals with laminectomy without cross-clamping); Group III (SCI with cross-clamping (Yasargil FE 721); Group IV (SCI with cross-clamping and intraperitoneal administration of carnosine 150 mg/kg and at the first hour and then at 6 h dose regimen of C); Group V (SCI with cross-clamping and ip administration of MP 30 mg/kg) at the first hour and 6 h dose regimen; and Group VI (SCI with cross-clamping and ip administration of C 150 mg/kg) and MP (30 mg/kg) at the first hour and at 6 h dose regimen of C and MP given in the same dose infusion. MP (Meng et al., 2011; Vaquero et al., 2006) and C (Di Paola et al., 2011) doses were chosen according to previously published data in other rat animal experiments. Surgery animals were anesthetized with an ip injection of ketamine (100 mg/kg). The end-point of an anesthesia was tested by toe pinch and eye blink reflexes, and the area for operation was disinfected and shaved. The animals were placed in a stereotaxic apparatus, and a midline incision made dorsally from T8 to T10. After carefully exposing the thoracic vertebrae (Fig. 1), a 70 g closing-force aneurysm clip (Yasargil FE 721, Aesculap, Istanbul, Turkey) was used to laminectomize the spinal cord for 1 min. After surgery, the animals were kept in a temperature-controlled incubator to be kept completely awake. Neurological evaluation was made by using the Tarlov scoring system (0 = no voluntary movement, spastic paraplegia; 1 = perceptible movement of the hind limbs; 2 = good movement but unable to stand; 3 = able to stand and walk; 4 = complete recovery (able to stand, walk and run)) as described previously (Shi et al., 2005; Tarlov and Klinger, 1954). After SCI the rats were observed for 24 h with a camera to see motor movements. Other experiment procedures are detailed elsewhere (Farooque et al., 1999).

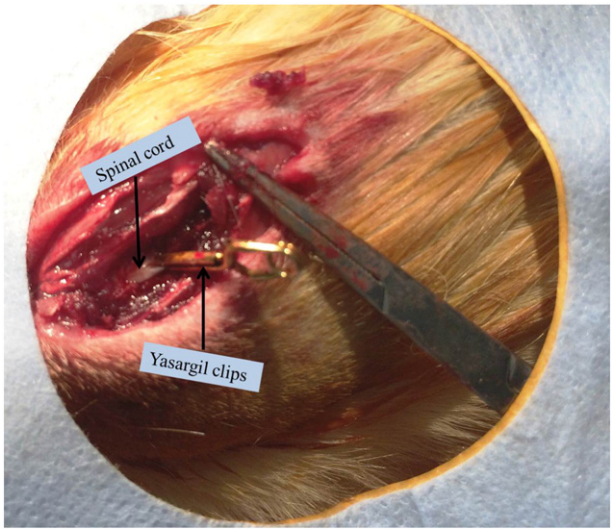

Fig. 1. Representative picture of laminectomy at T8–T10 with exposure of the spinal cord.

2.1. Plasma collection and tissue harvesting

At 24 h after overnight fasting, the rats were anesthetized by ip injection of 100 mg/kg ketamine until dead. Half of the blood samples were taken for biochemical analysis, the other half to measure irisin after collection into tubes with EDTA and 500 kallikrein inhibitor units (KIU) of aprotinin to prevent protease activity. Sample were centrifuged at 4000 rpm at 4 °C and the plasma separated, taken into Eppendorf tubes and stored at −80 °C until analysis. Brain and spinal cord tissues were rapidly removed and fixed in 10% formaldehyde solution for immunohistochemical analysis.

2.2. Immunohistochemistry

After preparing 4–5-μm sections, immunohistochemical analysis was done at room temperature as previously reported (Aydin et al., 2014b; Hsu et al., 1981). Deparaffinized tissue sections washed in running water for 5 min were passed through a graded alcohol series. After rinsing, incubation was in 3% H_2O_2 in absolute methanol for 5 min (Hydrogen Peroxide Block, TA-125-HP, Lab Vision Corporation, USA). Non-specific staining was blocked with normal goat serum (NGS). Immunohistochemical staining of brain and spinal cord tissues was carried out using the anti-irisin antibodies (1/200 ratio; H-067-17, Phoenix Pharmaceuticals, Inc., California, USA) as the primary antibodies and biotinylated goat anti-polyvalent (anti-mouse/rabbit IgG) and TP-125-BN (Lab Vision Corporation, USA) as secondary antibodies. Streptavidin–peroxidase treatment was added (TS-125-HR, Lab Vision Corporation, USA) for 30 min. Labeling was visualized by development with 3-amino-9-ethylcarbazole (AEC) substrate + AEC chromogen (AEC Substrate, TA-015 and AEC Chromogen, TA-002-HAC, Lab Vision Corporation, USA). Between steps, the slides were washed in PBS for 5 min and distilled water before being counterstained in Harris hematoxylin, dehydrated, and mounted with glycerol for inspection with an Olympus BX 50 photomicroscope. The staining was scored by both its intensity and prevalence on a scale of 0 to +3 (0: absence, +1: weak, +2: medium, +3: strong). Histological assessment with hematoxylin staining was done as previously described (Aydin et al., 2014b), using the following classification (0: absence, +1: weak, +2: medium, +3: