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Possible effects of some agents on the injured nerve in obese rats: A stereological and electron microscopic study☆

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ABSTRACT

Purpose: The main aim of this study was to research new treatments following peripheral nerve injury involving melatonin (Mel), acetyl-L-carnitine (ALCAR), and leptin (Lep) using updated unbiased methods at the stereological and electron microscopic levels.

Materials and methods: Wistar albino rats were randomly divided into nine equal groups; control (Cont), obese control (OG), obese group exposed to sciatic nerve resection (Gap) (OGG), obese group injected intraperitoneally (i.p.) with Mel (OMG), obese group injected with Mel i.p. with gap (OMGG), obese group injected with Lep i.p. (OLG), obese group injected with Lep i.p. with gap (OLGG), obese group injected with ALCAR i.p. (OAG), and obese group injected with ALCAR i.p. with gap (OAGG). Electromyography (EMG) procedures were performed. Following routine histological procedures, stereological analysis was performed for each group.

Results: In terms of the number of myelinated axons, high significant increase in OGG was observed compared to OG and Cont ($p < 0.01$). In addition, a highly significant increase in axon surface area and myelin thickness of OGG compared to OG and Cont ($p < 0.01$) was noted. A significant decrease in myelin thickness/axon diameter ratio of OGG was found in comparison with the other groups. In terms of latency, there was a highly significant decrease in OGG compared to Cont and OG ($p < 0.01$). Myelinated axon numbers in OAGG, OMGG and OLGG increased highly significantly compared to other groups ($p < 0.01$). Latency in OMGG, a highly significant increase, was determined in OMG compared to Cont ($p < 0.01$). In addition, latency values in OGG were highly significantly greater than in OAC and OAGG ($p < 0.01$).

Conclusion: In particular, administration of Lep, Mel and ALCAR as neuroprotective agents may make a positive contribution to regeneration and myelination in obese rats.

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1. Introduction

Studies of the peripheral nervous system (PNS) show that significant axonal regeneration is possible after axonal injury (Yiu and He, 2006). When an axon is damaged, the distal part undergoes Wallerian degeneration and loses its myelin sheath. In contrast, the proximal segment undergoes a repair process for regeneration. Although recovery rates vary depending on the damaged region under pathophysiological conditions, functional recovery usually occurs if axonal integrity is preserved (Lundborg, 2004; Allodi et al., 2012). During adulthood, there is a high potential for peripheral nerve regeneration. If axonal integrity cannot be preserved, morphological alterations such as in axon diameter and myelin sheath thickness in regenerated nerve fibers cannot be restored to normal pre-trauma levels (Muratori et al., 2012). Research is therefore required regarding possible neuroprotective agents that may potentially increase axonal regeneration following peripheral nerve injury.

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Obesity is a major and growing health problem. It is the result of energy uptake exceeding expenditure, and is characterized by chronic caloric imbalance. Obesity is therefore a complex disease. Many factors, such as genetic factors, environmental effects, metabolism, daily habits, culture and socioeconomic status play key roles in obesity. The prevalence of obesity, which is also induced by some drugs and neurobiological diseases, is increasing day by day (Naggert et al., 1997; Knecht et al., 2008). Insufficient studies regarding peripheral nerve regeneration and the role of obesity in this are available. However, Bekar et al. (2014) suggested that obesity might adversely affect regeneration after injury (Bekar et al., 2014). Additionally, and in agreement with that suggestion, Miscio et al. (2005) reported that obesity may lead to peripheral neuropathy and decreasing levels of cyclic adenosine monophosphate (cAMP) in the peroneal and tibial nerves (Miscio et al., 2005). Our study may help to elucidate the role of obesity in peripheral nerve regeneration and with the evaluation of treatment options.

Melatonin (Mel) is a neurohormonal regulator for circadian rhythm and body temperature and an antioxidant, the neuroprotective effects of which have been described in recent studies (Leon et al., 2005; Kaplan et al., 2011; Aygun et al., 2012; Lopez-Iglesias et al., 2014; Shinozuka et al., 2013). Mel secretion usually occurs at night and also continues until morning. It is synthesized from serotonin via a two-step pathway. When Mel is administered exogenously, it easily passes the blood–brain barrier and reaches high concentrations in the brain. Mel exhibits neuroprotective effects due to such characteristics as free-radical scavenging and lipophilic and hydrophilic properties (Aygun et al., 2012). Studies have suggested that Mel significantly prevents deoxyribonucleic acid (DNA) damage and neuronal apoptosis by reducing oxidative stress (Feng et al., 2006; Pandi-Perumal et al., 2006). The use of Mel in peripheral nerve regeneration related to nerve injury may therefore be beneficial because exogenous Mel administration significantly improves the antioxidant defense mechanism (Chang et al., 2008).

Leptin (Lep) has also been emphasized as a neuroprotective agent in recent years, as well as a hormone-regulating appetite suppressant. Lep is mainly secreted from adipose tissue, and from most tissues including skeletal muscle, gastric mucosa, placenta, and choroid plexus (Auwerx and Staels, 1998; Sandoval and Davis, 2003). Plasma Lep concentrations are generally proportional to the mass of fat tissue in the body (Wilding, 2001). Lep exhibits anti-obesity effects by reducing fatty acid oxidation, thus resulting in a decrease in fat tissue mass. Recent studies have demonstrated the neuroprotective and neurogenetic effects of Lep (Avraham et al., 2011; Perez-Gonzalez et al., 2011; Folch et al., 2012; Zhang et al., 2013). Exogenous Lep administration reduces neuronal damage following ischemia and stroke. Neuroprotective and neurogenetic effects of Lep on neuronal and glial cells by means of the development of and increases in neural stem cells have been reported in some studies (Avraham et al., 2011; Perez-Gonzalez et al., 2011).

Acetyl-L-carnitine (ALCAR) is a potential mitochondrial antioxidant with a particular neuroprotective effect against neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases (Serna et al., 2008; Chrysostomou et al., 2013). This mitochondrial agent also affects the degree of stem cell differentiation and reduces adipogenesis while stimulating osteogenesis and chondrogenesis (Lu et al., 2015). ALCAR can pass the blood–brain barrier using the cation carnitine transporter (OCTN2) and plays a key role in the oxidation of free fatty acids (Smeland et al., 2012). Studies have reported that all the neuroprotective effects of ALCAR occur through an increase in intracellular neurotrophic pathways or cholinergic neurotransmission (Bigini et al., 2002; Ori et al., 2002).

Recovery of peripheral nerve injury is possible using neuroprotective agents (Cheng et al., 2013). The main aim of the present study was to investigate new treatment options for peripheral nerve injury in obese rats in terms of neuroprotective agents, using updated and unbiased methods at the stereological and electron microscopic levels.

2. Materials and methods

2.1. Experimental design

The Experimental Animal Studies Ethics Committee of Ondokuz Mayıs University approved the study under approval no. 2010/121. Rats were obtained from the Experimental Animal Research and Application Center of the Ondokuz Mayıs University Medical Faculty (Samsun, Turkey). Fifty-four female Sprague Dawley rats aged 8–10 weeks and weighing 150–200 g were used. Animals were randomly divided into nine equal groups; control (Cont), obese control (OB), obese group exposed to sciatic nerve resection (Gap) (OGG), obese group injected with Mel intraperitoneally (i.p.) for 21 days (50 mg/kg/day; Sigma–Aldrich, St Louis, MO, USA) (OMG), obese group injected with Mel i.p. with gap for 21 days (OMGG), obese group injected with Lep i.p. for 21 days (1 mg/kg/day; Sigma–Aldrich, St Louis, MO, USA) (OLG), obese group injected with Lep i.p. for 21 days with gap (OLGG), obese group injected with ALCAR i.p. for 21 days (50 mg/kg/day; Sigma–Aldrich, St Louis, MO, USA) (OAG) and an obese group injected ALCAR i.p. for 21 days with gap (OAGG). All rats were housed in plastic cages and maintained in appropriate temperature and humidity conditions (22 ± 2 °C, 50 ± 5%) in a 12-h light/12-h dark cycle. Experimental groups (OB, OGG, OMG, OMGG, OLG, OLGG, OAG, and OAGG) were fed a special diet with a 40% fat. However, standard pelleted rat feed was used in this experiment for a control group (Altunkaynak et al., 2008). The rats in the experimental and control groups were weighed once a week and alterations in weight of rats were noted individually. The average food consumption was reported daily for each rat. During the experiment, the body weight gain of each rat was noted by calculating the difference between the final weight and the initial weight. According to these data, at the end of 8 weeks, body mass indexes (BMI) of rats were calculated to determine whether the rats were obese. The final BMI values of the rats were calculated as final weight (kg) divided by square of the length from the nose to the anus (m^2). The same methods were used in accordance with other studies for adiposity measurements. The rats with a BMI more than 5 kg/m^2 were considered obese (Ersoy and Çakır, 2007; Altunkaynak et al., 2008).

2.2. Surgical procedures

All surgical procedures were performed after i.p. injection of ketamine (Ketasol 90 mg/kg, Richter Pharma AG, Weis, Australia) and xylazine (Rompun 10 mg/kg, Bayer, Leverkusen, Germany). The right legs were shaved and the gluteal regions incised with a thin incision under deep anesthesia. The biceps femoris muscle was dissected, and the sciatic nerve was removed. A collagen membrane (in Epigui, Riemser Inc., Germany) was placed approximately 10 mm above the nerve branch. Resection resulted in a 5-mm gap. The gap region was then completely surrounded and closed by a collagen membrane in tube form. Standard gaps were thus produced for each rat (Fig. 4).

2.3. Electrophysiological analysis

EMG procedures were performed shortly before the rats in all groups were sacrificed on the 90th day at the Ondokuz Mayıs

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