Neuroscience Letters 561 (2014) 123–127

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The effects of resveratrol on chronic constriction injury of sciatic nerve in rats

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HIGHLIGHTS

- Resveratrol treatment prevented locomotor impairment in chronic constriction injury of rat sciatic nerve.
- Resveratrol treatment improved histomorphological alterations caused by chronic constriction injury of sciatic nerve.
- Protective effects of resveratrol may be mediated through the restoration of IGF-1 levels.

ARTICLE INFO

Article history:
Received 9 November 2013
Received in revised form
19 December 2013
Accepted 23 December 2013

Keywords:
Resveratrol
Chronic constriction injury
Locomotor function
Morphometry
Insulin-like growth factor 1
Rat sciatic nerve

ABSTRACT

The aim of this study was to investigate the effects of resveratrol (RVT) in chronic constriction injury (CCI) of sciatic nerve by behavioral, histomorphological and immunohistochemical evaluations in rats. In this study, male Wistar rats were divided into three groups: sham ($n = 7$), CCI + saline ($n = 7$) and CCI + RVT ($n = 7$). After inducing CCI, treatment with 10 mg/kg/day of RVT or saline for 14 days was given. Locomotor function was assessed with rota-rod and open field tests. Morphologic alterations of sciatic nerve were assessed histologically by light and electron microscopy. Immunohistochemistry for insulin-like growth factor-1 (IGF-1) were performed. RVT treatment prevented motor impairment and histomorphological alterations caused by chronic constriction injury of sciatic nerve. IGF-1 immunoreactivity was significantly higher in RVT treated group then CCI induced group and positive correlated with morphometric parameters. These results indicate that RVT may reduce CCI induced damage and this effect may be mediated through the restoration of IGF-1 immunoreactivity.

1. Introduction

Peripheral nerve damage, which causes sensory and motor disorders, is found commonly in the population. Surgical repairs of peripheral nerve constriction injury as well as the different treatment methods are also being tested. Peripheral nerve injury leads to many cellular and molecular changes in neurons and glial cells. Peripheral neuropathies due to nerve damage may occur as secondary to diseases such as cancer and diabetes, or postoperative complication of surgery or trauma [17,18,26,29]. In order to understand the mechanisms underlying in neuropathic nerve damage, several experimental models are used. Chronic constriction injury (CCI) animal model is the most frequently model due to create findings similar to those seen in humans [13,15].

Several molecular mechanisms are thought to contribute to the development of CCI. It has been demonstrated that sciatic nerve injury induces change of neurotrophic factors expressions such as nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) and other trophic factors [10,28]. Insulin-like growth factor-1 (IGF-1) is a potent neurotrophic factor that is widely expressed in central nervous system and essential for brain growth and development [7,19]. Insulin-like growth factors have been shown to enhance the survival of motor neurons and the rate of peripheral nerve regeneration [6,22].

Resveratrol (3, 4, 5 trihydroxystilbene, RVT) is a naturally occurring phytoalexin present in high concentration in foods such as grapes and wine [12]. It has been demonstrated that RVT has antioxidant and neuroprotective properties. Protective effects of RVT have been showed in brain, heart and intestine ischemia models [2,20,23]. In spinal cord injury model, the effect of RVT on IGF-1

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0304-3940/$ – see front matter.
http://dx.doi.org/10.1016/j.neulet.2013.12.056

signaling in rat femurs has been demonstrated [27]. However, the effects of RVT on locomotor function, sciatic nerve morphometry and IGF-1 immunoreactivity have not been studied in CCI of rat sciatic nerve. Thus the aim of this study was to investigate the effects of RVT in CCI of sciatic nerve by behavioral, histomorphological and immunohistochemical evaluations in rats.

2. Materials and methods

2.1. Animals

Twenty-one male Wistar rats weighing 200–250 g were used in this study. They were housed in polycarbonate cages with food and water ad libitum. All experimental protocols were approved by the Ethics Committee of Animal Care and Experimentation of the University of Dokuz Eylul, Turkey.

2.2. Chronic constriction injury and experimental groups

Chronic constriction injury was induced in rats as previously described in the method of Bennett and Xie [4]. The common sciatic nerve was exposed at the middle level of left thigh by blunt dissection through the biceps femoris muscles. Four ligatures (silk 4/0) were placed loosely around the nerve proximal part of the sciatic's trifurcation at a distance of 1 mm between each ligature. The ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed. After performing nerve ligation, muscular and skin layer was immediately sutured with thread and topical antibiotic was applied at once. Similar procedures were applied to the sham group, except that nerve was exposed but not ligated.

The rats were divided into three groups; sham group ($n = 7$), CCI + saline group ($n = 7$) and CCI + RVT group ($n = 7$). CCI + RVT group was injected with Resveratrol (R5010, Sigma) 10 mg/kg ip once a day for 14 days, starting the 1st day after CCI [8]. CCI + saline group was injected with vehicle (5% ethanol in saline) ip for 14 days. At the end of the experiment, the rats in each group were sacrificed by cervical dislocation under ether anesthesia. The sciatic nerve samples were taken out for light and electron microscopic assessment.

2.3. Rota-rod test

At day 14th post-surgery, motor coordination was evaluated using the rota-rod test. This apparatus consists of a base platform and a rotating horizontal rod (7 cm in diameter, 50 cm in length) divided into three separate compartments. The rod was set to accelerate from 5 to 40 rpm in a 5 min period. Each rat was given 3 training sessions before testing. During the test session, the latency (s) for the first fall during a 5 min period was observed.

2.4. Open field test

The open-field consisted of a 1 m × 1 m area surrounded with 50 cm high wall, with a video camera installed 2.5 m above the apparatus. At day 14th post-surgery, each rat was placed in the center of the open-field and then locomotor activity (ambulation) was measured for 5 min in a soundproof observation room illuminated with controlled light (100 lx). The recording and analysis of the locomotor activity were done using the HVS and Noldus-Ethovision-XT video tracking systems. Number of entries in the middle cells of open-field-arena and walking distance in open-field- arena were measured in this assay.

2.5. Histological evaluation

Distal parts of the crushed site of the left sciatic nerves were sampled for every group. Samples were fixed in 2% paraformaldehyde plus glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) and stored at +4 °C for minimum 24 h. They were postfixed with osmium tetraoxide in a 0.1 M sodium phosphate buffer for 90 min at room temperature and subsequently contrasted with 0.5% uranyl acetate in 70% acetone and 1% phosphotungstic acid overnight at +4 °C. The pieces were processed by routine method and embedded in araldit. The specimens were then cross-sectioned on an ultra-microtome (Leica Ultracut R). Semithin sections were examined and photographed by a light microscope (Olympus CX41) while the thin sections were examined by a transmission electron microscope (Carl Zeiss Libra 120 EFTEM). Axon counts, axon diameter and myelin thickness were calculated using the Image J program. For morphometric analysis, three sections from each rat were randomly selected. Ten randomly selected areas per section were digitally photographed at 40× magnification and calculation process was performed using a counting frame with 2500 μm^2 from nerve cross-section. All measurements were carried out in a blinded fashion.

2.6. Immunohistochemistry

For immunohistochemical staining, nerve samples were processed by routine histological methods and embedded in paraffin blocks. The immunohistochemistry procedure for IGF-1 (1/100, bs-0081R, Bioss, USA) was performed on paraffin sections. The sections were incubated overnight with anti-IGF-1 antibody and then for another 30 min with the biotinylated mouse secondary antibody. The bound secondary antibody was then amplified with Histostain® plus bulk kit (Invitrogen, 85–9043). The antibody–biotin–avidin–peroxidase complexes were visualized using DAB. The sections were finally mounted onto lysine-coated slides. Three sections were used for each sample in performing the immunohistochemical scoring. The qualitative intensity of staining for IGF was assessed using a scale between 0 and 3. With 0 representing no detectable stain, 1; mild, 2; moderate and 3 representing strong stain.

2.7. Statistical analysis

Differences in the behavioral and morphological parameters between the groups were analyzed using the one-way-ANOVA post hoc Bonferroni test. Correlations among groups were calculated using Pearson correlation analysis. Results are presented as mean ± SEM and data with IGF-1were given as median (IQR) (significance level was $p \leq 0.05$).

3. Results

In rota-rod test; partial ligation of sciatic nerve produced motor impairment compared to control rats. Chronic constriction injury of sciatic nerve significantly impaired motor performance with marked reductions in mean latencies to fall off the rota-rod. RVT treatment prevented motor impairment caused by chronic constriction injury of sciatic nerve (Fig. 1, $p < 0.05$).

In the open-field test, the rats with chronic constriction injury of sciatic nerve demonstrated a decrease in the walking distance compared to control rats. The administration of RVT was significantly increased the walking distance (Fig. 2A). A decrease in the number of entries into center cells was observed in CCI + saline rats compared to control group. Total entries into center cells were increased in CCI rats treated with RVT (Fig. 2B, $p < 0.05$). The decrease in the walking distance and total number of entries revealed a decrease in locomotor activity in CCI rats.

Morphometric analysis of sciatic nerves revealed that the axon number, axon diameter and myelin thickness in CCI + saline group was significantly decreased compared to sham group ($p < 0.05$).