Journal of Neurological Sciences [Turkish] **32:(2)**# 44; 303-310, 2015
http://www.jns.dergisi.org/text.php3?id=882
Research Article

Effects of Resveratrol on Acute Sciatic Nerve Injury in a Rat Model

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Summary

Aim: This study evaluated the therapeutic efficacy of resveratrol (RVT), a naturally occurring polyphenol, during the management of acute nerve injury in a rat model.
Materials and Methods: A total of 28 rats were divided into trauma, control and low- and high-dose RVT groups (all n = 7). In all three non-control groups, trauma was induced by a medium-pressure aneurysm clip, applied to the sciatic nerve for 30 s. After trauma, the animals were treated with RVT for 7 days, after which nerve tissue samples were obtained from the site of injury for use in histological and stereological analyses.
Results: There was a significant group difference in myelin thickness. At 50 mg/kg, RVT treatment was associated with increased myelin thickness, but a decrease was observed at 100 mg/kg.
Conclusion: RVT has a beneficial effect on acute crush injury of the sciatic nerve at a dose of 50 mg/kg.

Key words: Sciatic nerve, resveratrol, myelin thickness, axon diameter

Deneysel Sıçan Modelinde Akut Siyatik Sinir Hasarında Resveratrol'ün Etkisi

Özet

Amaç: Doğal bir fenol olan resveratrol'ün (RVT) deneysel sıçan modelinde akut sinir hasarı tedavisinde terapötik etkinliğini araştırmaktır.
Gereç ve yöntemler: Toplamda 28 sıçan dört gruba ayrılmıştır; travma, kontrol, düşük doz RVT ve yüksek doz RVT (n=7). Kontrol grubu dışındaki diğer gruplarda orta-basınçlı anevrizma klibi ile 30 saniye boyunca sinir hasarı oluşturuldu. Travma sonrası 7 gün boyunca RVT tedavisi verildi. Sonrasında, hasar yerinden alınan sinir dokusu örnekleri histolojik ve steriyolojik analizlere tabi tutuldu.
Bulgular: Myelin kalınlığı yönünden gruplar arasında anlamlı farklılık saptandı. 50 mg/kg RVT ile tedavinin myelin kalınlığını artırdığı bununla birlikte 100 mg/kg dozunda myelin kalınlığında azalma olduğu tespit edildi.
Sonuç: RVT siyatik sinir akut ezilme yaralarında 50 mg/kg dozunda faydalı etkiye sahiptir.

Anahtar Kelimeler: Siyatik sinir, resveratrol, myelin kalınlığı, akson çapı

INTRODUCTION

Despite its apparently increased incidence, consensus on the optimal management of acute peripheral nerve injury has not been reached. The principal objective of acute wound management is preventing the transition to chronic injury by ensuring sufficient tissue oxygenation for the

optimal functioning of fibroblasts, which play a primary role in wound healing[8,19]. Various drugs have been used to achieve this aim, and identifying novel agents for the treatment of peripheral nerve injury remains an active area of research[15,19,20].

Resveratrol (3,5,4 trihydroxystilbene; RVT) is synthesized from coumaroyl CoA and malonyl CoA by the enzyme RVT synthase in response to stress, injury, infection or UV irradiation. RVT, which exhibits significant antioxidant properties, is neuroprotective against excitotoxicity, ischemia and hypoxia both in vitro and in vivo[7,9,12,14,21,23]. A recent study demonstrated improvements in locomotor performance and histomorphology in response to RVT treatment in a rat model of chronic sciatic nerve constriction[3]. However, no study has evaluated the effects of RVT on acute nerve injury. We investigated herein the acute histological and stereological effects of RVT on sciatic nerve crush injury in a rat model.

MATERIAL AND METHODS

The study was performed according to American Guidelines for the Ethical Care of Animals following approval from the Ethics Committee of Yüzüncü Yıl University. A total of 28 albino adult female Wistar rats (8–10 weeks old; weight = 250 ± 20 g each) were purchased from Charles River Laboratories (Wilmington, MA, USA). The animals were housed in a regulated laboratory environment, according to a 12-h light/dark cycle, and had free access to food and water. They were divided into four groups of seven animals, all of whom were weighed before the experiment and at sacrifice. For anesthesia, 8 mg/100 g ketamine (Alfamine 10%; Ege Vet Hayvancılık Bornova-İzmir, Alfasan International BV, Woerden, The Netherlands) and 1 mg/100 g xylazine (Alfazyne 2%; Ege Vet Hayvancılık Bornova-İzmir, Alfasan International BV) were administered intraperitoneally. Surgery was performed on the left sciatic nerve in all animals.

In the control group, only sciatic nerve dissection was performed; in the acute crush injury group, sciatic nerve dissection was performed after clamping for 30 s with a medium-pressure aneurysm clip. In the third group, low-dose RVT (50 mg/kg/day [Lot: 12494; Expiration date: O06/14]; Santa Clara, CA, USA) was injected for 7 days intraperitoneally after acute sciatic nerve crush injury and dissection; in the fourth group, high-dose RVT (100 mg/kg/day) was used. On day 8, the sciatic nerves in all groups were excised 5 mm proximally and distally from the lesion.

Histology and Stereology

During stereological analysis, the left sciatic nerve of each animal was exposed and a 4-mm-long nerve segment was carefully removed. The segments were cut into blocks of equal length followed by fixation with 2.5% glutaraldehyde buffered in 0.1 M cocodilate (pH 7.4) for 20 h. After fixation, the tissues were rinsed twice in cocodilate buffer. The specimens were then postfixed in 1% osmium tetroxide for 1.5 h. Peripheral nerves were dehydrated in an ascending alcohol series and washed twice with propylene oxide. The tissues were then embedded in epoxy resin. For light microscopy, semi-thin sections (750 nm) obtained using a Leica Ultracut UCT ultramicrotome (Wien, Austria) were stained with toluidine blue solution (1% toluidine blue and 2% borate in distilled water)[17,18]. A stereological analysis of peripheral nerves was performed by researchers blinded to group in accordance with the method of Turgut et al.[17]. One section was obtained from each peripheral nerve. An unbiased counting frame (2500 μm^2) was utilized. Area sampling of peripheral nerve sections was achieved with a 1/6 proportion using systematic random sampling. All axon profiles were sampled regardless of their shape or cross-sectional area. An Olympus BX53F light microscope (Tokyo, Japan) and CCD color

video camera (JVC, Tokyo, Japan) were used (magnification: 1000×, 100× oil objective lens, numerical aperture: 1.25). The total number of axons in each peripheral nerve was estimated by multiplying the counted number of axons by the reverse of the area fraction. The optical dissector/cavalieri combination was used to minimize the effects of technical artifacts and section thickness on the results (Figures 1–2)[11].

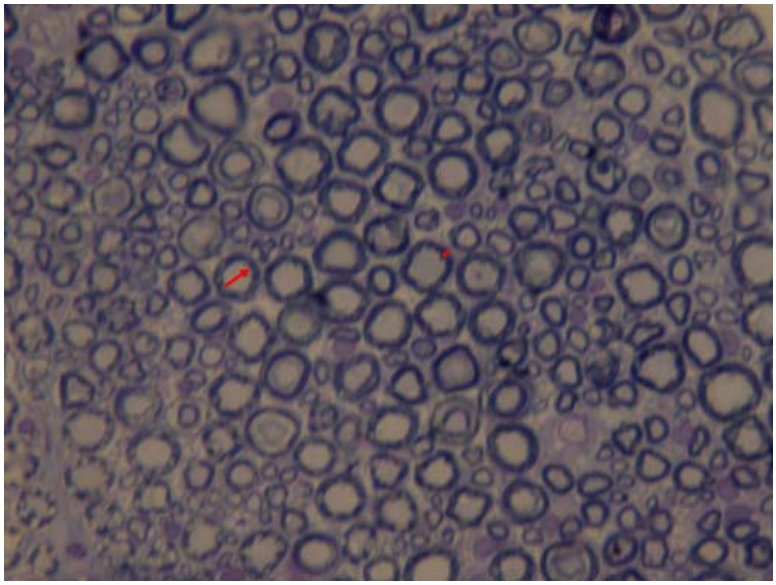

Figure 1: *Micrographs of sciatic nerve cross-sections (→ : axon diameter; ¯: myelin thickness).*

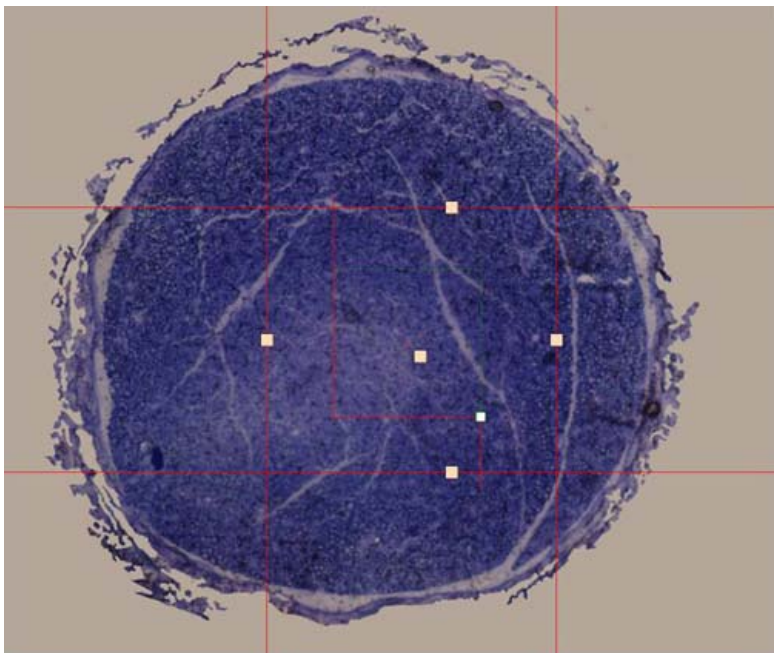

Figure 2: *Profile area of the sciatic nerve (10× magnification).*

The following formula was applied: $N = \bar{Q}^{-} x \sum P \times k \times \frac{a/p}{a\,(frame)^2}$

$N = \bar{Q}\text{-}x\sum P \times k \times$, where N is the total axon density, $\bar{Q}$- is the mean number of axons, $\sum P$ is the total number of points, k is the section sequence, a/p is the point area, and a(frame) is the counting frame area.

Statistical Analysis

Statistical analyses were performed using the SPSS for Windows software package (ver. 18.0; SPSS Inc., Chicago, IL, USA). Parametric values are provided as means ± standard deviation, with non-parametric values listed as percentages. To compare continuous parametric variables, Student's t-test was used. The Mann-Whitney U test was used to compare continuous nonparametric variables. Categorical data were compared using the chi-square test. Two-tailed p-values of <0.05 were taken to indicate statistical significance.

RESULTS

The mean axon diameter was 4.41 µm (range: 4–5.15 µm), 3.63 µm (range: 3.20–3.86 µm), 4.15 µm (range: 2.85–5.40 µm) and 4.37 µm (range: 3–5.35 µm) in the trauma, control and low- and high-dose RVT groups, respectively. There was a significant difference in mean axon diameter between the trauma and control groups (p = 0.002), possibly due to the edematous effect of trauma. A significant difference was also observed between the trauma and low-dose RVT groups (p = 0.002), but there were no differences between any of the other groups (p>0.05).

The mean myelin thickness was 1.62 µm (range: 1.45–1.75 µm), 1.27 µm (range: 1–1.70 µm), 1.89 µm (range: 1.45–2.27 µm) and 1.52 µm (range: 1.25–2 µm) in the trauma, control and low- and high-dose RVT groups, respectively. There was a significant difference in mean myelin thickness between the trauma and control groups (p = 0.001), possibly due to the edematous effect of trauma, and between the control and low-dose RVT, low- and high-dose RVT, trauma and low-dose RVT (all p = 0.004), control and high-dose RVT and trauma and high-dose RVT groups (both p = 0.005).

The axon density was 742.14 µm (range: 384–956 µm), 517.85 µm (range: 242–887 µm), 657.42 (range: 268–992 µm) and 670.14 (range: 304–985 µm) in the trauma, control and low- and high-dose RVT groups, respectively. There were no significant group differences in axon density (p>0.05).

In a histological comparison of the control and trauma groups, greater vacuolization and degeneration in the myelin sheath was observed in the trauma group and in the high- vs. low-dose RVT group (Figures 3 - 6).

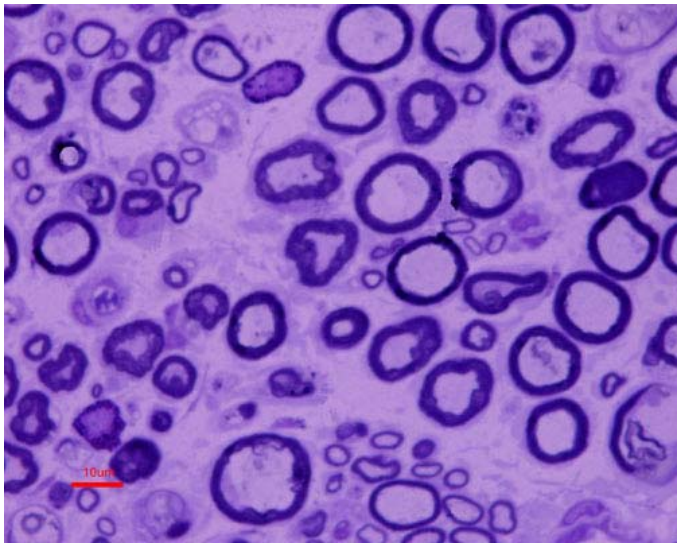

Figure 3: *Micrographs of sciatic nerve trauma cross-sections. Vacuolization and degeneration were detected in the myelin sheath. Toluidine blue staining; scale bar = 10 µm.*

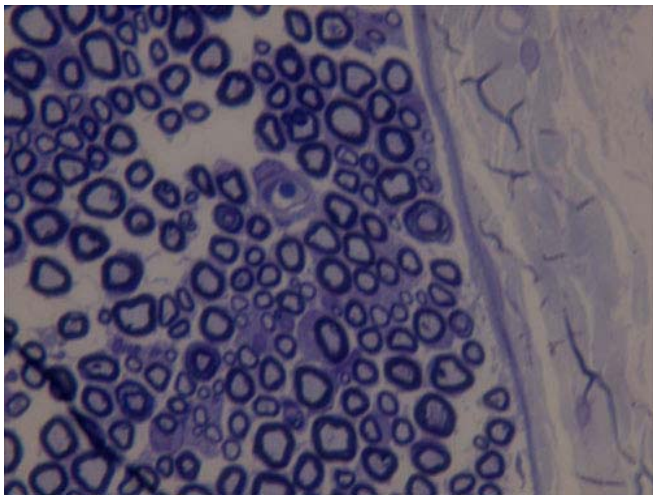

Figure 4: *Micrographs of sciatic nerve cross-sections in the control group with myelin sheaths. Toluidine blue staining (100× magnification, oil immersion lens).*

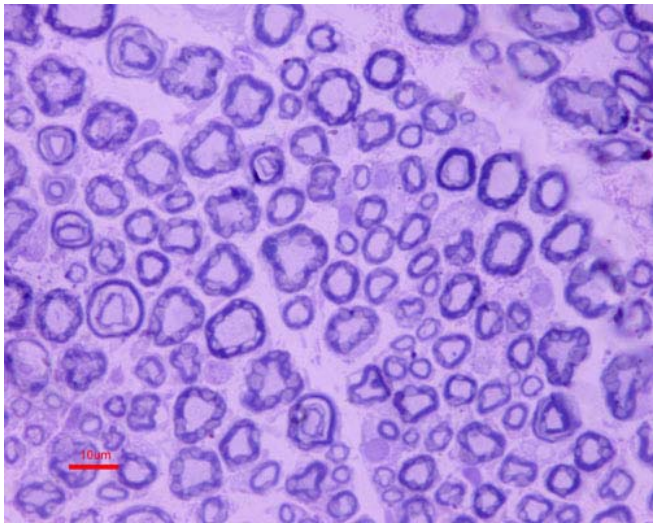

Figure 5: *Micrographs of sciatic nerve cross-sections in the low-dose (50 mg) resveratrol group. Vacuolization and degeneration were detected in the myelin sheath. Toluidine blue staining; scale bar = 10 µm.*

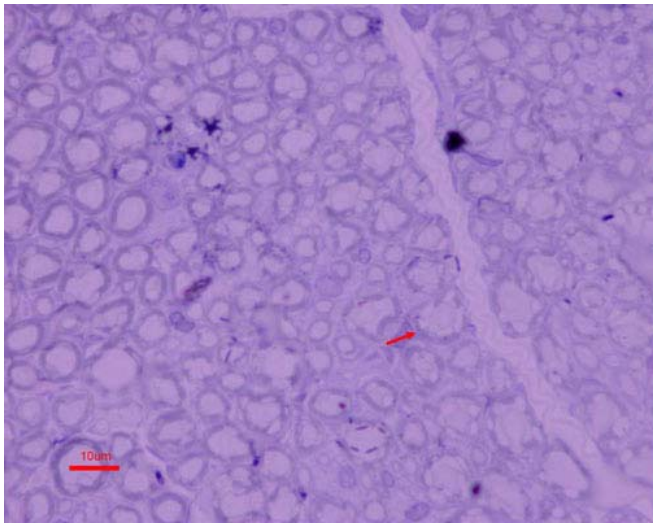

Figure 6: *Micrographs of sciatic nerve cross-sections in the high-dose (100 mg) resveratrol group. Vacuolization and degeneration were detected in the myelin sheath (→ indicates vacuolization). Toluidine blue staining; scale bar = 10 µm.*

DISCUSSION

We demonstrated herein a therapeutic role for RVT, indexed by increased myelin thickness at a low (50 mg/kg) dose, in an acute sciatic nerve crush injury model.

RVT, a naturally occurring polyphenol present in grape juice, wine, peanuts, pistachios, blueberries and bilberries[3,10,13], exerts physiological and pharmacological effects, including anti-oxidative, neuroprotective, antiplatelet agglomerative, blood lipid metabolism

modulatory, anti-inflammatory and anti-tumor growth effects[3,10,13,22]. A protective role for RVT has also been documented in epilepsy, cerebral ischemia and neuronal degeneration models; RVT also protects the spinal cord from ischemia-reperfusion injury[1,10]. In a rat model of spinal cord injury, Liu et al.[10] demonstrated the neuroprotective and functional recovery effects of RVT; the authors speculated that these effects were due to the anti-oxidative, anti-inflammatory, and apoptotic properties of RVT.

Neurons are highly susceptible to oxidative injury due to their high level of oxygen consumption. Several studies have demonstrated that RVT may protect the brain and spinal cord against traumatic neuronal injury following contusion or ischemia-reperfusion injury by modulating the activity of neuronal markers of oxidative stress at the site of injury. RVT decreases lactate dehydrogenase, xanthine oxidase, metalloproteinase 9, heme oxygenase, and malondialdehyde levels, and it attenuates the reduction in glutathione levels induced by several types of injury[2,4,13,16]. Other possible mechanisms of action of RVT include differential expression of nitric oxide synthase, inhibition of peroxisome proliferator-activated alpha receptor and decreased expression of NFκB p65 during inflammation after ischemia-reperfusion injury[6,13].

Although its actions in the brain and spinal cord have been studied extensively, there are few reports on the effects of RVT in the peripheral nervous system. Recently, Bağrıyanık et al.[3] investigated the behavioral, histomorphological and immunohistochemical effects of RVT during chronic constriction injury of the sciatic nerve. RVT reduced chronic constriction injury-induced damage, an effect possibly mediated by its restoration of IGF-1 immunoreactivity. However, data are lacking regarding the acute effects of RVT against acute injury. In this study, we sought to assess these effects in a dose-dependent manner using a simple and well-established rat model of acute sciatic nerve crush injury in which injury was induced in <1 min. RVT conferred acute therapeutic effects during peripheral nerve injury at 50 mg/kg, but was toxic at 100 mg/kg.

The optimal management strategy for acute nerve injury remains subject to debate. The therapeutic effects of various agents have been described, including alpha lipoic acid and vitamin C in combination with mannitol and nicergoline[15,19,20]. In two different studies, nerve tissue samples were obtained only 1 h and 7 days after trauma[15,20]. In peripheral nerve injury studies, axonal diameter, myelin thickness and the number of axons were used to assess nerve regeneration[3,5]. Similarly, we measured axon diameter, myelin thickness and axonal density in crushed nerve tissue using histomorphological and stereological techniques. In all of these studies, the principal aim was to prevent an acute injury from progressing to a chronic state. Our data suggest that RVT is effective in this respect, provided that an appropriate dose is used.

The present study is limited by its use of a rodent model that included a relatively small number of animals. Furthermore, the mechanism underlying the activity of RVT during acute sciatic nerve injury remains unclear, although a previous chronic injury study suggested that it involves the restoration of insulin-like growth factor-1 immunoreactvity[3]. Despite these limitations, our data exhibit promise with respect to the treatment of a challenging clinical condition.

In conclusion, RVT confers beneficial effects during acute sciatic nerve injury at a dose of 50 mg/kg. However, to delineate the mechanistic pathways underlying this beneficial effect, further research is required.

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Received by: 17 March 2015
Revised by: 12 April 2015
Accepted: 24 May 2015

The Online Journal of Neurological Sciences (Turkish) 1984-2015
This e-journal is run by Ege University Faculty of Medicine,
Dept. of Neurological Surgery, Bornova, Izmir-35100TR
as part of the Ege Neurological Surgery World Wide Web service.
Comments and feedback:
E-mail: editor@jns.dergisi.org
URL: http://www.jns.dergisi.org
Journal of Neurological Sciences (Turkish)
Abbr: J. Neurol. Sci.[Turk]
ISSNe 1302-1664

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