

1-1-2020

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Recommended Citation

ÇETİNKAYA, AYHAN and ÇAMSARI, ÇAĞRI (2020) "Effects of carvacrol administration on motor function following spinal ischemia and reperfusion," *Turkish Journal of Zoology*. Vol. 44: No. 5, Article 5.

<https://doi.org/10.3906/zoo-2006-18>

Available at: <https://journals.tubitak.gov.tr/zoology/vol44/iss5/5>

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Effects of carvacrol administration on motor function following spinal ischemia and reperfusion

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Received: 11.06.2020 • Accepted/Published Online: 22.08.2020 • Final Version: 14.09.2020

Abstract: The sensitivity of the medulla spinalis to ischemia and reperfusion has been shown in previous in vitro and in vivo studies. However, less is known about the effects of carvacrol administration following ischemia and reperfusion. It was hypothesized that carvacrol might have protective effects on motor neuron function under ischemia- and reperfusion-induced oxidative stress. A total of 24 adult Wistar rats were divided into three groups: group I (control group; n = 8), group II (spinal ischemia and reperfusion group; n = 8), and group III (spinal ischemia and reperfusion + carvacrol group; n = 8). Ischemia and reperfusion were performed by clamping the abdominal aorta for 45 min. Clamps were then removed and 100 mg/kg of carvacrol was administered to group III. In the control groups a vehicle solution was administered. Animals were then observed for motor deficit index 48 h following the ischemia. Prior to the termination of the experiment, blood serum was obtained through intracardiac puncture for analyses of total antioxidant status and total oxidative stress levels. The results did not show effects on total antioxidant status or total oxidative stress levels. However, the motor deficit index was significantly different between the carvacrol and spinal ischemia groups and between the control and spinal ischemia groups. Our study demonstrated improved motor function in spinal ischemia models following carvacrol administration. However, future studies are required to determine the mechanism which improves motor function under carvacrol administration.

Key words: Spinal ischemia, carvacrol, ratoxidative stress, motor deficit index

1. Introduction

The medulla spinalis is one of the organs most sensitive to ischemia. Spinal ischemia and reperfusion (SIR) is a common pathological condition which occurs following arterial occlusion and many thoracoabdominal surgeries and includes trauma-induced ischemia, clamping, transplantation, and surgical shock (Gökce et al., 2016; Lianget al., 2011; Tokmak et al., 2015). Ischemia is inevitable following aortic clamping. Due to reduced blood flow, neurological complications and the formation of reactive oxygen species (ROS) cause damage to the central nervous system, which includes the medulla spinalis. The prevalence of spinal ischemia is associated with many factors, including surgical operation techniques (time and management of aortic clamping, operative technique, reconstruction, etc.), age, and patient-related factors. The range of occurrence is between 2.9% and 38% (Badem et al., 2014; Korkmaz et al., 2015). Decreasing ischemic times, reducing spinal cord swelling, and diminishing postoperative monitoring of neurological functions are some of the clinical applications for spinal ischemia

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prevention. However, none of these applications are sufficient to eliminate the problem (Cemilet al., 2016; Lianget al., 2011; Shaafiet al., 2011).

Increased oxidative stress is very common in spinal cord injuries due to a cascade of inflammation and the production of free radicals (Bedreag et al., 2014). Moreover, the increased production of free radicals also causes an altered blood flow that has adverse clinical impacts (Bedreaget al., 2014; Crimi et al., 2012; Kayacan et al., 2019). There are many in vivo and in vitro studies examining improvement in loss of neurological function and attempts to reduce oxidative stress following SIR (Badem et al., 2014; Cemil et al., 2016; Erkut and Onk, 2015; Gokce et al., 2016a; Gökce et al., 2016b; Korkmaz et al., 2015; Liang et al., 2011; Shaafi et al., 2011; Usulet al., 2008; Tokmak et al., 2015). However, no studies have examined the effects of carvacrol on SIR. Carvacrol (CAR) is a monoterpenic phenolic compound (2-methyl-5-Isopropylphenol) belonging to the Lamiaceae family (Ozturket al., 2018; Yu et al., 2012), which is naturally found in many plants (Suonet al., 2014). Carvacrol is frequently

used by the food industry as a food additive in desserts, beverages, and as a sweetener in chewing gums (Fenaroli, 1975). There are many studies in the literature reporting its antithrombic (Enomoto et al., 2001), antimicrobial (Ben Arfa et al., 2006), antifungal (Ahmad et al., 2011; Dambolena et al., 2011), and antitumor (Can Baser, 2008) effects. These effects of carvacrol have been shown in many ischemia and reperfusion studies using kidney, liver, and gastrointestinal tissues (Canbek et al., 2008; Ozturk et al., 2018; Suo et al., 2014).

Carvacrol administration following SIR was reported to inhibit acetylcholinesterase (AChE) activity in Alzheimer's disease (Orhan et al., 2008; Jukic et al., 2007; Kaufmann et al., 2011). Likewise, it was reported to show antidepressant activity through the dopaminergic system. Because of these activities, we hypothesized that CAR has a protective effect in SIR models. In the present study, our aim was to investigate the protective effects of CAR on neurologic functions under SIR-induced oxidative stress.

2. Materials and methods

The study protocol was approved by the local ethics committee of Bolu Abant İzzet Baysal University (decision number:2018/2; May 22, 2019). The study was conducted in the Laboratory of Animal Research and Application Center. Twenty-four adult Wistar albino rats were randomly divided into 3 groups (n = 8 animals per group). They were followed for 2 weeks, and no pathological conditions were determined. Animals were kept in a controlled environment with 12 h light:12 h dark conditions and food and water provided ad libitum. The animal laboratories were maintained at 19–21 °C with 55%–60% relative humidity using heating and ventilation systems (ÜNTES, Ankara, Turkey).

2.1. Preparation and administration of carvacrol

Carvacrol (Sigma Aldrich, St Louis, MO, USA) at a dose of 100 mg/kg was administered to rats intravenously (iv).

2.2. Anesthesia and surgical procedure

The animals were anesthetized intramuscularly (IM) with 90 mg/kg of ketamine and 10 mg/kg of xylazine. The depth of anesthesia was confirmed by assessing the respiratory rate and reflex tones. Under sterile conditions, the abdominal area was shaved, and a 3 cm incision was made at the linea alba line. The abdominal aorta was dissected proximal to the bifurcation region, which is distal to the renal arteries. Following 45 min of ischemia, clamps were removed in groups II and III. One hundred mg/kg of carvacrol was administered to group III. In the control group a vehicle solution (0.9% NaCl) was administered, and the surgical area was stitched. The animals were observed for motor deficit index (MDI) 48 h following the ischemia. Afterward, 5 mL of blood was obtained through intracardiac puncture under anesthesia before the animals were sacrificed. Serum

was obtained following 10 min of centrifugation at 4000 rpm, and total antioxidant status (TAS) and total oxidative stress (TOS) were determined (Figure 1).

2.3. Study groups

Group I (control group; n = 8): Abdominal incision was performed at the linea alba line, and then the incision was stitched.

Group II (spinal ischemia and reperfusion group; n = 8): Motor deficit index was determined 48 h following SIR treatment for 45 min.

Group III (spinal ischemia and reperfusion + carvacrol group; n = 8): Spinal ischemia was performed for 45 min, and animals were administered CAR throughout reperfusion. Their MDI was determined 48 h following reperfusion.

2.4. Determination of motor deficit index

Motor deficit index was determined 48 h following SIR, as described in the literature (Hwang et al., 2017). Motor deficit index is defined as the sum of ambulation and stepping reflex, where an MDI value of six indicates the highest neurological damage. Evaluation parameters of MDI are provided in Table 1.

2.5. Evaluation of total oxidative status

Total oxidative status was determined using ELISA, based on manufacturer instructions (Rel Assay Diagnostics, Gaziantep, Turkey) (Yeni et al., 2005). For the procedure, in brief, 45 µL of standard solution or blood serum were placed in 96-well plates and mixed with 300 µL of reagent I. Following the determination of the first absorbance (A1) at a wavelength of 530 nm, 15 µL of reagent II were added to each well. The mixture was incubated at room temperature for 10 min, and the second absorbance (A2) was determined at a wavelength of 530 nm. The results were calculated based on the formula below:

$$A2 - A1 = \Delta Abs \text{ (for either a standard or a sample)}$$

$$\text{Result} = [\Delta Abs_{\text{sample}} / \Delta Abs_{\text{standard}}] * 10$$

2.6. Evaluation of total antioxidant status

Total antioxidant status was determined using ELISA, based on manufacturer instructions (Rel Assay Diagnostics, Gaziantep, Türkiye) (Erel, 2004). In brief, 18 µL of standard or blood serum were added to each well of 96-well plates and mixed with 300 µL of reagent I. Following the determination of first absorbance (A1) at a wavelength of 660 nm, 45 µL of reagent II were added. The samples were incubated at room temperature for 10 min, and second absorbance (A2) was determined at a wavelength of 660 nm. The results were calculated based on the formula above.

3. Statistical analysis

Motor deficit index median values were determined in each group. The differences in MDI results were calculated using SAS 9.2 statistical software (SAS Institute, Cary, NC, USA), and the difference between groups was

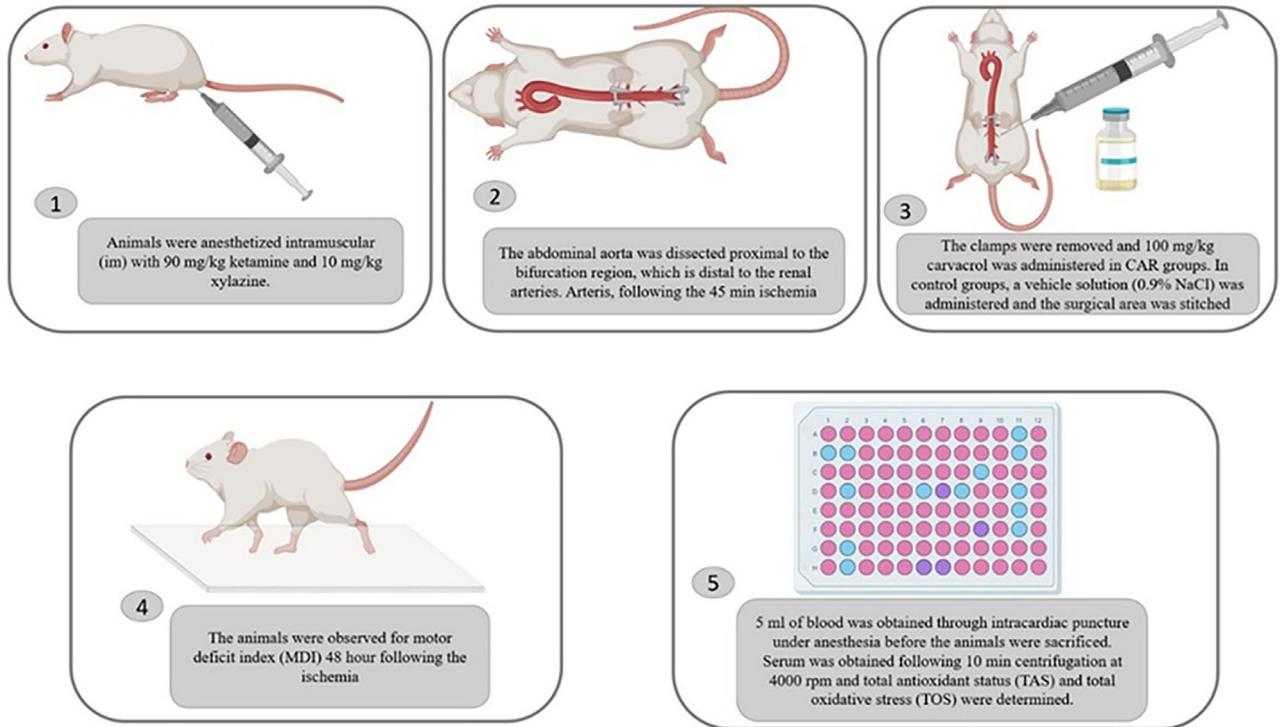


Figure 1. Anesthesia and surgical procedure.

Table 1. Evaluation parameters of motor deficit index.

Walking with the rear limbs	Stepping reflex
0: Normal	0: Normal stepping reflex
1: Toes grasp the ground normally, but ataxia is present.	1: Poor reflex
2: Toes look lumpy when moves.	2: No step
3: Extremities are mobile, but cannot even perform the lumpy move.	
4: No movement. Dragging the extremities.	

determined by Kruskal–Wallis test. When a difference was found between groups, the Mann–Whitney U-test was performed. The differences in total oxidative stress and total antioxidant status were evaluated using one-way ANOVA with the General Linear Models module (Proc GLM) in SAS. When there was a significant difference in ANOVA, Dunnett’s multiple comparison test was applied to evaluate the difference between the least square means for the control versus treatment groups. Statistical significance was $P < 0.05$, and data was presented as mean \pm standard error of the mean.

4. Results

4.1. Motor deficit index:

Median values of MDI are provided in Table 2.

In the MDI results there was a significant difference

between the CAR and spinal ischemia groups and between the control and spinal ischemia groups ($P < 0.05$).

4.2. Total oxidative stress

No significant difference was observed between the groups ($P > 0.05$; Figure 2A).

4.3. Total antioxidant status

There was no significant difference observed between the groups ($P > 0.05$; Figure 2B).

5. Discussion

In the present study, the effects of carvacrol administration on MDI following SIR were investigated. Motor deficit index data evaluations suggested that administering CAR 48 h after an injury reduced motor function capacity (MFC). Many studies in the literature showed that MFC was an important parameter for the evaluation of SIR

Table 2. The values are represented as the median of each group.

Experimental group	Group (n)	Ambulatory (point)	Stepping reflex (point)
Control	8	0.0	0.0
Sham control	8	1.0	0.0
Spinal ischemia	8	2.0	0.0
Carvacrol	8	0.5	0.0

(Badem et al., 2014; Erkut and Onk, 2015; Korkmaz et al., 2015; Liang et al., 2011). Some studies used motor function evaluation methods similar to those used in the current study (Ryu et al., 2018). Other studies used different motor function evaluation methods (Badem et al., 2014; Erkut and Onk, 2015; Guven et al., 2015).

In Korkmaz et al. (2015), which investigated the effects of CysLT1 receptor antagonist montelukast following SIR, MDI was evaluated for MFC scoring. In other studies, MFC scoring following SIR was performed by Tarlov scale (Erkut and Onk, 2015; Guven et al., 2015) or Basso, Beattie, and Bresnahan (BBB) scoring (Basso et al., 1995; Basso et al., 1996), which uses 21-point scoring to determine the activity of an animal. Basso, Beattie, and Bresnahan scoring was used for the evaluation of rear limb motor dysfunction tests following spinal injury (Basso et al., 1996). Alternatively, the Tarlov scale was used to evaluate animals within five different grades following reperfusion. These were as follows; grade 0: total paralysis, grade 1: minimal movement at the articulations, grade 2: standing without support, grade 3: standing alone, grade 4: weak jumping, and grade 5: completion (Tarlov, 1972). The efficiency and reliability of MDI scoring has been shown in previously published studies.

The similar effects of CAR and MFC following SIR suggested that their effect is mediated through motor neurons. However, future studies are required to demonstrate the intracellular and extracellular pathways used by this plant extract.

Carvacrol administration following spinal ischemia and reperfusion did not change total antioxidant status or total oxidative stress levels. The formation of free radicals was expected after ischemia and reperfusion. In SIR studies, ROS measurements were performed in samples obtained from serum or the medulla spinalis. In a study performed by Jian et al. (Fuet al., 2018), the administration of luteolin in an SIR model caused significant effects on malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px). Likewise, in another study (Guvemet al., 2015), superoxide dismutase and malondialdehyde demonstrated significant differences. In previously published studies, the administration of

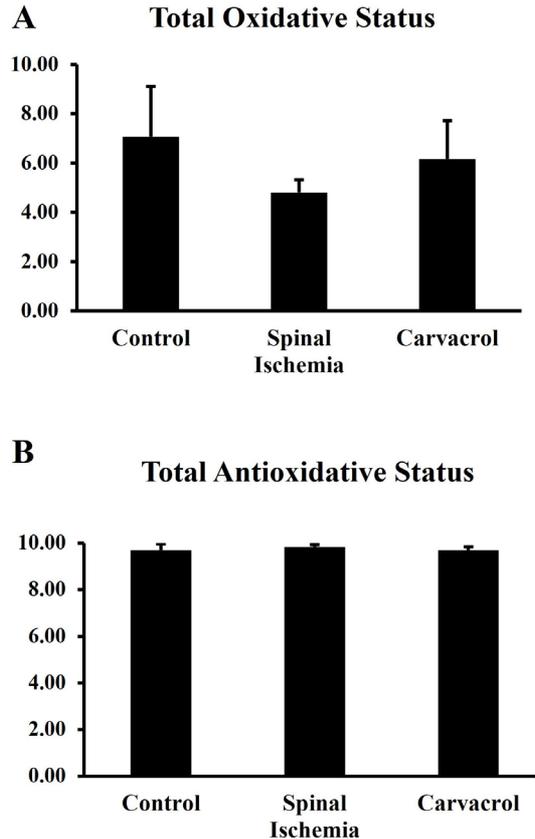


Figure 2. The schedule of total oxidant and antioxidant values of groups.

different plant extracts reportedly had no effect on MDA, SOD, GSH-Px, and myeloperoxidase (Badem et al., 2014).

6. Conclusion

In conclusion, the results of the present study suggest that carvacrol administration could improve motor function following SIR. However, more studies are required to examine the effects of carvacrol administration on pathophysiological mechanisms following SIR.

Acknowledgment

The figures for this article were created with the aid of BioRender.

Conflict of interest

The authors have no conflicts of interest to declare with respect to the authorship and/or publication of this article. Figures for this paper were created with the aid of BioRender.

Funding

The authors received no financial support for the research for and/or authorship of this article.

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