

Effects of Pregabalin on Kidney Tissue in Spinal Cord Ischemia Reperfusion Injured Rats

Spinal Kord İskemi Reperfüzyon Hasarı Oluşturulan Ratlarda Pregabalinin Böbrek Dokusu Üzerine Etkisi

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ABSTRACT

Objectives: The purpose of this study was to investigate the possible protective effects of low and high dose pregabalin that was administered in rat in a spinal cord ischemia-reperfusion (I/R) study model.

Material and Method: We used 24 Wistar albino rats as subjects in our study. They were divided into 4 groups; randomized Control (C group), I/R (I/R group), I/R-low dose (30 mg/kg) pregabalin (I/R-LP group) and I/R-high dose (200 mg/kg) pregabalin (I/R-HP group). All groups have undergone a laparotomy intervention under anesthesia. In I/R group, a cross clamp was placed in the abdominal aorta just after the laparotomy for 120 minutes (to cause spinal cord ischemia injury) and then reperfusion was achieved by opening the vascular clamp. At the end of the study, kidney tissue was obtained for determining total oxidant status (TOS) and total antioxidant status (TAS) levels, histochemical and immunohistochemical determination.

Results: Total Oxidative Status (TOS) enzyme activity was significantly higher in I/R group when compared to the control, I/R-LP and I/R-HP groups. Likewise, Total Antioxidant Status (TAS) enzyme activity was remarkably higher in I/R group when compared with the C, I/R-LP and I/R-HP groups. VEGF staining has yielded no expression in renal tissues. In microscopical analysis of the tissue slides which were immunohistochemically stained with p53 antibody, some crucial findings have been established as follows: As p53-expressing cells were not detected in the control group, the presence of p53-expressing cells were clearly identified at different intensities in several bowman capsules in the I/R group. However, no expression was detected in general tubules. Interestingly, p53 expression levels were prominently lower in low-dose pregabalin given group and considerably higher in the 200 mg/kg pregabalin administered group, which was more pronounced than the I/R group.

Conclusion: Results established from the current study suggest that pregabalin given at different doses may have a partial protective effect in kidney tissues of rats undergone experimental spinal cord IR injury.

Keywords: Spinal cord ischemia-reperfusion, pregabalin, kidney, p53, TOS, TAS, histopathology.

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ÖZET

Amaç: Bu çalışmanın amacı, spinal kord iskemi-reperfüzyon (I/R) çalışma modelinde ratlara uygulanan düşük ve yüksek doz pregabalinin olası koruyucu etkilerini araştırmaktır.

Yöntem: Bu çalışmada 24 adet Wistar cinsi albino rat kullanıldı. Ratlar rastgele 4 gruba ayrıldı. Kontrol (K grubu), I/R (I/R grubu), I/R-düşük doz (30 mg/kg) pregabalin (I/R-LP grubu) ve I/R-yüksek doz (200 mg/kg) pregabalin (I/R-YP grubu). Tüm gruplara anestezi altında laparotomi girişimi uygulandı. I/R grubunda laparotomiden hemen sonra abdominal aortaya 120 dakika (spinal kord iskemi hasarına neden olmak için) mikrovasküler klemp konuldu ve ardından vasküler klemp açılarak reperfüzyon sağlandı. Çalışma sonunda total oksidan durum (TOS) ve total antioksidan durum (TAS) düzeylerinin belirlenmesi, histokimyasal ve immünohistokimyasal belirleme için böbrek dokusu elde edildi.

Bulgular: Total Oksidatif Durum (TOS) enzim aktivitesi, kontrol, I/R-LP ve I/R-HP grupları ile karşılaştırıldığında I/R grubunda anlamlı olarak daha yüksek bulundu. Benzer şekilde, Total Antioksidan Durum (TAS) enzim aktivitesi, I/R grubunda K, I/R-LP ve I/R-YP gruplarına göre anlamlı yüksek olarak tespit edildi. VEGF boyaması ile böbrek dokularında ekspresyon sağlanmadı. P53 antikoruna ile immünohistokimyasal olarak boyanmış dokuların mikroskopik analizinde aşağıdaki gibi bazı önemli bulgular tespit edilmiştir: Kontrol grubunda hiçbir p53 ekspresyon eden hücre bulunmazken I/R grubunda çok belirgin ve bilhassa bazı bowman kapsülleri içinde farklı yoğunluklarda p53 ekspresyonu tespit edildi. Genel tübüllerde herhangi bir ekspresyon tespit edilmedi. İlginç bir şekilde, düşük doz pregabalin verilen grupta p53 ekspresyon seviyeleri belirgin şekilde daha düşüktü ve 200 mg/kg pregabalin uygulanan grupta I/R grubundan bile daha belirgin olarak yüksekti.

Sonuç: Mevcut çalışmadan elde edilen sonuçlar, farklı dozlarda verilen pregabalinin deneysel spinal kord İR yaralanması geçirmiş ratların böbrek dokularında kısmi koruyucu bir etkiye sahip olabileceğini düşündürmektedir.

Anahtar Sözcükler: Spinal kord iskemi-reperfüzyonu, pregabalin, böbrek, p53, TOS, TAS, histopatoloji

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INTRODUCTION

Spinal cord I/R process triggers complex pathologic cascades, which in turn can lead to serious neuronal destruction via cellular damage, necrosis or apoptosis mechanisms. It is well known that the oxidative stress resulting from the depletion of endogenous antioxidant defense systems and the excessive production of ROS play an important role in the formation of I/R induced damage, as the pathogenic mechanisms of spinal cord I/R damage are not fully understood. Lipid peroxidation of cell membranes caused by these RORs and inflammatory cytokine production further aggravate secondary neuronal damage (1). Kidney tissue is very sensitive against I/R damage due to its high energy requirement and dense vascular network. Renal I/R injury is associated with delayed graft function following transplantation, shock complication, and increased mortality/morbidity as a complication cardiac or aortic surgery (2).

Lungs and kidneys are initially affected from I/R-induced damage. Besides hepatic, myocardial and neuronal functions may also be deteriorated, which in turn may lead to a multiple organ failure (3). Pregabalin is a structural analogue of gamma-aminobutyric acid (GABA). Voltage-sensitive calcium channels (VGCC) which have anti-convulsant, analgesic and anxiolytic effects in animal models are highly potent ligands of the "alpha-2-delta" subunits. After strong binding of pregabalin to the alpha-2-delta subunit of the calcium channels localized on the cell membranes of hypersecretory neurons, the depolarization-induced calcium flux reduces the release of many excitatory neurotransmitters including glutamate, noradrenaline and substance-P (4). Anti-apoptotic and anti-inflammatory effects of pregabalin in spinal cord injury-induced rats have also been reported (5). Effect of pregabalin in oxidative stress and inflammatory processes in various tissues is a still ongoing research field. In this regard, it would be a useful approach to apply medicines that could help the surgeon's job by reducing the damage and prolonging the clamping time while protecting the patient. There are numerous drugs and antioxidant substances which have been studied and investigated in treatment of ischemia reperfusion injury. In recent years, different approaches have been achieved to prevent kidney I/R injury in clinical trials. In the current study, we aimed to investigate the possible beneficial effects of pregabalin on renal damage in rat spinal cord I/R injury model, in which protective effect of some tissues on I/R injury has already been indicated (1).

MATERIALS and METHODS

Animals and Experimental Protocol

This study was carried out in GUDAM Laboratory of Gazi University with the approval of the Experimental Animals Ethics Committee of Gazi University (18/02/2016 GÜET-16.016) and whole procedures have been fulfilled in accordance with the standards in the Guide for the Care and Use of Laboratory Animals. A total of 24 male Wistar Albino 12 weeks old rats weighing between 200 and 300 grams were included to the study. Animals were housed in standard cages in a pathogen free environment under a constant 12 h dark-light cycle for at least one week prior to surgery. They had free access to food (until 2 h before the anaesthetic procedure) and water during this time period.

Aortic Occlusion and Ischemia Reperfusion Model

Intramuscular ketamine hydrochloride (100 mg/kg; Ketalar; Parke-Davis; Pfizer, Inc., New York, NY, USA) and xylazine hydrochloride (Alfazyne, 2%; Ege Vet, Ltd., Izmir, Turkey) (Ketalar[®] vial, Parke-Davis, USA) injection was applied for anesthesia procedure. The intervention has been achieved under a heating lamp, while the rats were fixated in the supine position. Midline laparotomy was performed on rats whose skin was prepared aseptically. Following removal of the intestines with a wet gauze, infrarenal abdominal aorta was explored. An atraumatic microvascular clamp was placed in the abdominal aorta. After 120 minutes, the abdominal aortic microvascular clamp was removed and reperfusion was obtained for 120 minutes. Aortic ischemia and reperfusion with loss of pulsation in the distal aorta during clamping were confirmed by the return of the pulmonary artery to the distal aorta after removal of the clamp. Only laparotomy and abdominal aortic dissection were performed in control group with equal time relapses of 240 mins without I/R. Peritoneal cavity was treated with saline and the caudal incision temporarily closed with a wet gauze in order to minimize the loss of heat and fluid from the peritoneal cavity during the I/R process, abdominal aortic clamping and post-removal periods. Then, kidneys were removed and animals were sacrificed after two hours.

One of the kidneys was removed from each animal in such a way that its integrity would not be traumatized and fixated in neutral buffered formaline solution.

Experimental Group Procedure

Group Control (Group C, n=6): The spinal cord was laparotomized and closed without I/R injury, and rats were anesthetized 120 minutes.

Ischemia/Reperfusion (Group I/R, n = 6): Spinal cord I/R injury was performed in this group. Renal tissues of rats were anesthetized 120 minutes.

Ischemia/Reperfusion + Low Dose Pregabalin (Group I/R-LP, n=6): Rats were administered intraperitoneally 30 mg/kg pregabalin (Lyrica[®], Pfizer, USA) 15 minutes prior to ischemia. After laparotomy, a cross clamp was placed in the abdominal aorta for 120 minutes to perform spinal cord ischemia injury. Following 120 minutes of ischemia, reperfusion was achieved by opening the vascular clamp.

Ischemia/Reperfusion + High Dose Pregabalin (Group I/R-HP, n=6): This group was intraperitoneally administered pregabalin at 200 mg/kg dose 15 minutes before ischemia. After laparotomy, a cross clamp was placed in the abdominal aorta for 120 minutes to perform spinal cord ischemia injury. Following 120 minutes of ischemia, reperfusion was achieved by opening the vascular clamp. The left kidney was placed in neutral buffered formaline solution for histopathological evaluation and the right kidney was frozen in liquid nitrogen and stored at -80 ° C for biochemical analysis.

Histopathological Evaluation

Kidney tissue samples were processed, embedded in paraffin blocks, cut at five micrometers thickness with a microtome. Tissue slides were then stained with hematoxyline-eosin to examine the general tissue properties and Periodic Acid Schiff to evaluate the renal tubule and Bowman capsule basement membrane structures. Sections were also immunohistochemically stained with p53 and VEGF primer antibodies to assess the vascular exchange / inflammatory activation and whether the cell genome has been preserved and the cells were actively regenerated during the I/R process.

Homogenization of Tissues

Renal tissues were collected into a sterile eppendorf tube and kept at -80°C until being analyzed for total antioxidant/oxidant status. Without being allowed to dissolve, the tissues were quickly weighed on a precision scale and separated as 80-100 mg using a no. 22 lancet (PLUSMED[®]). Tissue fragments were smashed in the presence of liquid nitrogen in a porcelain bowl. The powdered tissue was transferred to the homogenization tube and for each gram of tissue, 140 mM KCl solution was added such that the dilution was 1/10. In order not to increase the temperature homogenization tube was held in a snow-filled glass beaker and after homogenization with a homogenizer (Glas_Col K5424[®]) at a speed of 50 revolutions per minute (rpm) for two minutes homogenization process was completed. Homogenate was transferred to eppendorf tube. Eppendorf tubes were coated with parafilm and then centrifuged (Hettich Micro 200R[®]) for 10 minutes at 3000 rpm. After centrifugation, the supernatant was taken into another eppendorf tube and TOS and TAS levels were measured.

Total Antioxidant Status

The TAS was measured by the TAS test kit (RelAssay Diagnostic[®], Turkey). For TAS measurement, as described in kit's procedure, 500 µL of reagent 1 (measurement buffer) and 30 µL of sample were mixed and absorbance was measured at 660 nm by a spectrophotometer (NanoDrop[®] ND-1000) (A1). 75 µL of reagent 2 (colored 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was added to the mixture in the Eppendorf tube. The tube was coated with paraffin and incubated for 5 minutes in a hot water bath at 37 °C (Lightning Labortechnik[®]). After incubation, absorbance measurement was performed at 660 nm (A2). For standard measurement, the Trolox Eq solution at a concentration of 1 mmol / L was used instead of the sample. The first and second measurements were made for three times and their averages were measured. The absorbance change (ΔAbs) was calculated by subtracting the first absorbance value (A1) from the second absorbance value (A2). TAS levels were calculated using the formula given in the kit and expressed as mmol Trolox Eq / L. $TAS = [(\Delta Abs_{H2O} - \Delta Abs_{Sample}) / (\Delta Abs_{H2O} - \Delta Abs_{Standard})]$

Total Oxidative Status

The TOS was measured by the TOS test kit (RelAssay Diagnostic®, Turkey). For TOS measurement, as described in kit's procedure, 500 µL of reagent 1 (measurement buffer) and 75 µL of sample were mixed and absorbance was measured at 530 nm by a spectrophotometer (NanoDrop® ND-1000) (A1). 25 mL of reagent 2 (Pro-chromogenic solution) was added to the mixture. The tube was coated with paraffin and incubated for 5 minutes in a hot water bath at 37 °C (Lightning Labor teknik®). After incubation, absorbance measurement was performed at 530 nm (A2). A standard solution containing 10 µmol / L hydrogen peroxide (H2O2) equivalent / liter given in the kit was used for standard measurement. The first and second measurements were made for three times and their averages were measured. The absorbance change (Δ Abs) was calculated by subtracting the first absorbance value (A1) from the second absorbance value (A2). TOS levels were calculated using the formula given in the kit and expressed as mmol H2O2 Eq / L. $TOS = [(\Delta Abs \text{ sample}) / (\Delta Abs \text{ standard})] \times \text{Standard Concentration (10 } \mu\text{mol / L)}$.

Statistical Analysis**Table 1.** Oxidative status parameters in renal tissues [Mean \pm SD]

	Group C (n=6)	Group I/R (n=6)	Group I/R+ LP (n=6)	Group I/R+HP (n=6)	p**
TOS (mmol H ₂ O ₂ Eq/l)	0.92 \pm 0.10*	1.18 \pm 0.15	1.03 \pm 0.13*	0.96 \pm 0.08*	0.005
TAS (mmol Trolox Eq/l)	38.23 \pm 5.27*	47.73 \pm 2.18	42.71 \pm 3.15*	39.31 \pm 4.19*	0.002

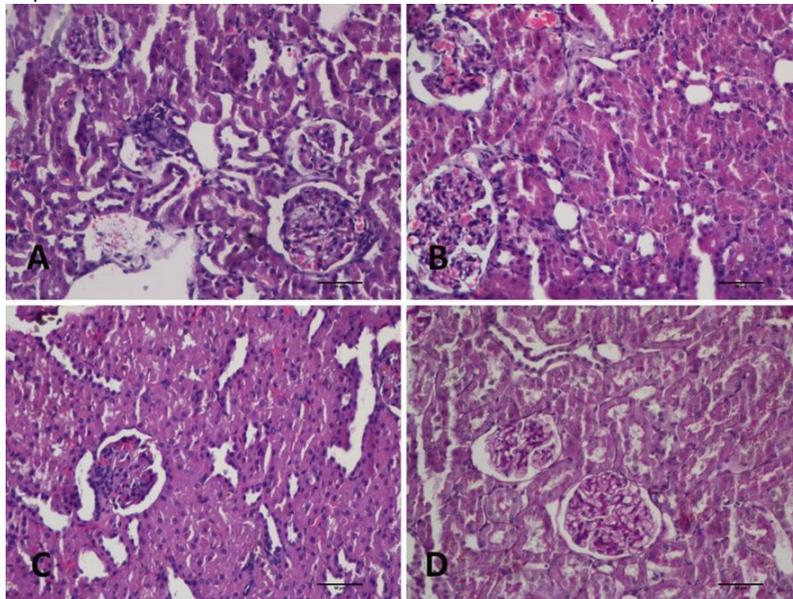
p**: Significance level with Kruskal Wallis test, p< 0.05

*p<0.05: Compared with Group I / R

Total Antioxidant Status: TAS

Total Oxidative Status: TOS

It has been observed that the amount of erythrocytes leaking out of the vessel walls in the Bowman capsule in the I/R group was higher than the control group in histopathological evaluation. That is, there was an edema in the Bowman capsule. In low-dose pregabalin given group, there was no change in this histopathological finding. Besides, the 200 mg/kg pregabalin given group has exhibited a significant improvement resembling the appearance of the controls. However no inflammatory cell migration, angiogenetic changes or neovascularization were observed (Figure 1A-D). Although PAS staining showed very slight edematous change in the I/R and low dose groups, there was no significant difference between the control and 200 mg/kg groups and there was no problem in the basal membrane in the inner vessels of the Bowman's capsule

**Figure 1:** Renal tissue images stained with hematoxyline-eosin.

(A: Control group, B: Ischemia reperfusion group, C: Ischemia reperfusion with low dose pregabalin administration group, D: Ischemia reperfusion with high dose pregabalin administration group)

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 20.0 program was used for the statistical analysis. The Bonferroni-adjusted test was used if the results of the One-way ANOVA test were significant to determine which groups differed from the others. The results were expressed as mean \pm standard deviation (mean \pm SD). Statistical significance was set at a p value of < 0.05.

RESULTS

There was a significant difference between the groups in terms of renal TOS enzyme activity (p = 0.005). TOS enzyme activity was significantly higher in I/R group than the I/R-LP and I/R-HP groups (p=0.001, p=0.039 and p=0.004, respectively) (Table 1). There was a significant difference between the groups (p=0.002) by means of TAS enzyme activity, which was significantly higher in I/R group than the I/R-LP and I/R-HP groups (p <0.0001, p = 0.036 and p= 0.001, respectively) (Table 1).

and in all other tubules (Figure 2 AD). VEGF staining has yielded no expression in renal tissues (Figure 3A-D). Whereas, p53 staining had important findings. When there was no p53 expressing cell in the control group, it was detected in I/R group very distinctly, especially at different intensities in some Bowman's capsule sites. However, no expression was detected in general tubule structures. Interestingly, it was found that p53 expression was markedly faded in low dose pregabalin given group but, very prominent in 200 mg/kg pregabalin applied group. The expression in this group was even more pronounced than in I/R group (Figures 4A-D).

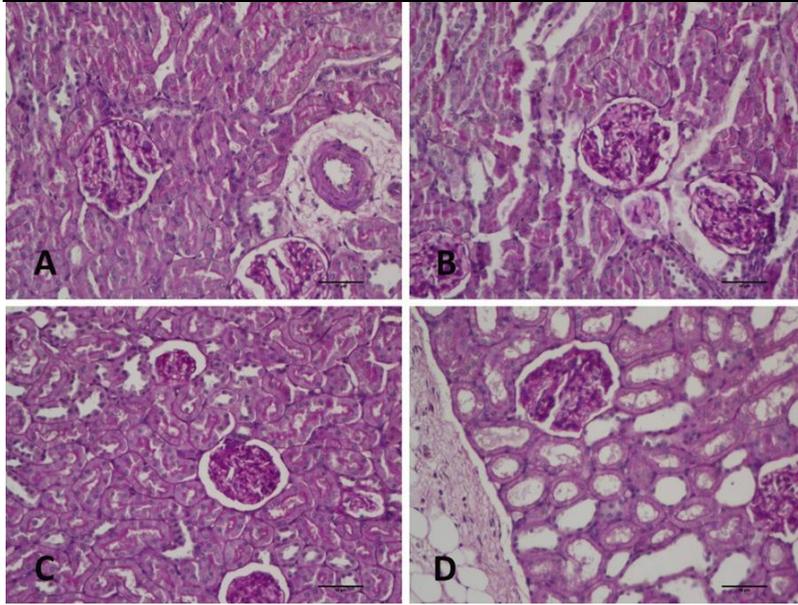


Figure 2. Results of kidney tissue PAS staining.

(A: Control group, B: Ischemia reperfusion group, C: Ischemia reperfusion with low dose pregabalin given group, D: Ischemia reperfusion with high dose pregabalin given group).

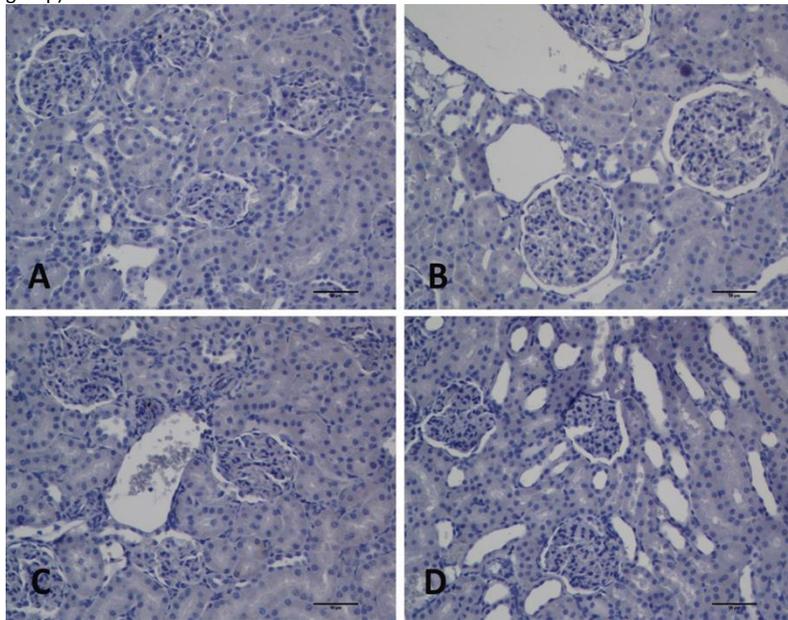


Figure 3. VEGF expression in kidney tissues.

(A: Control group, B: Ischemia reperfusion group, C: Ischemia reperfusion with low dose pregabalin group, D: Ischemia reperfusion with high dose pregabalin group).

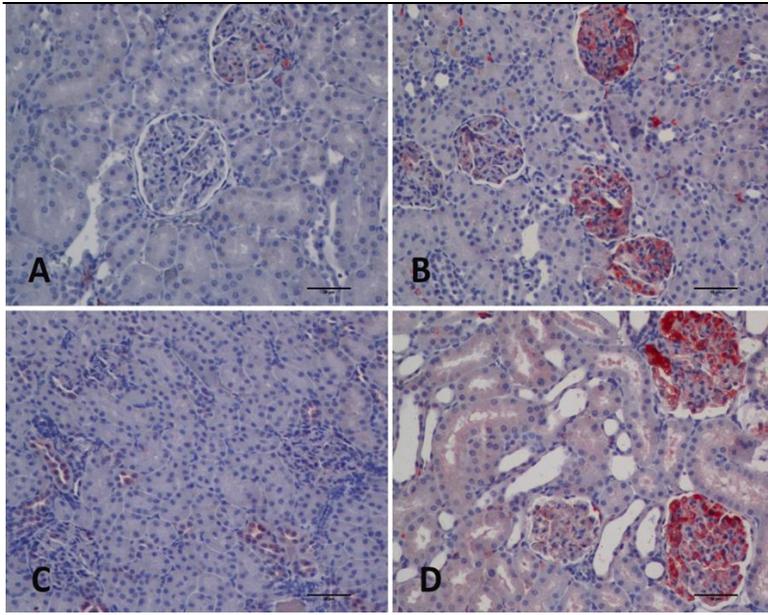


Figure 4: p53 expression in renal tissues.

(A: Control group, B: Ischemia reperfusion group, C: Low dose pregabalin given group with IR, D: High dose pregabalin given group with IR).

DISCUSSION

Spinal cord I/R injury is a destructive and unpredictable complication of thoraco-abdominal aortic interventions, which may lead to paraplegia in nearly 40% of the whole cases. Various approaches have been endeavored to prevent this catastrophic complication, but no effective therapeutic method could be developed so far. Although the pathophysiological mechanisms underlying the spinal cord I/R injury are not fully understood, it is well-known that paradoxical reperfusion-induced initial ischemic damage is caused by the neuronal tissue damage as a result of a pathological chain reaction involving glutamate-mediated exotoxicity, oxidative stress, lipid peroxidation, inflammation and apoptosis. Renal I/R injury is a deleterious clinical insult with unsolicited damage outcomes, which can be frequently encountered during various conditions including haemorrhagic shock, vascular surgery, partial nephrectomy intervention, accidental or iatrogenic traumas. The underlying mechanisms of kidney I/R injury are complex and unclear, however oxidative stress is an important contributor to the pathogenesis of renal I/R injury process. Acute oxidative stress response following the renal blood flow interruption and reperfusion gives rise to excessive ROS production, which may cause cell death by various means, including lipid peroxidation, DNA mutation and apoptotic or necrotic cascades (6). One of the most common causes of perioperative ABD is I/R injury.

Temporary decrease in blood flow to the kidneys leads to severe and permanent damage to the renal parenchyma once the blood flow restores (7). Renal tubular infrastructure is very sensitive to ischemia. Prolonged ischemic exposure leads to epithelial cell death especially in proximal sites of the renal tubules. Although this is the case, it is still possible to recover the normal tissue structure and functions of the kidney after exposure to long stools. Damaged kidney tissue usually heals following an I/R-induced inflammation process. In fact, the proinflammatory / antiinflammatory and damage / repair processes are closely intertwined during the rescue procedures following I/R-related injury (8). ROS, proinflammatory mediators increase, leukocyte infiltration, Ca⁺⁺ loading, phospholipid peroxidation and reduction are suggested as mechanisms responsible for ischemia reperfusion injury (9).

Pregabalin is a structural analog of GABA. Although there is a GABA structural analogue, there is no direct effect on GABA-like mechanisms. Pregabalin is a potent ligand for the "alpha-2-delta" subunit of VGCC, which has anti-convulsant, analgesic and anxiolytic effects in experimental animal models (10). After binding strongly to the alpha-2-delta subunit of calcium channels of hypersecretory neurons, depolarization-induced calcium flux and thus the release of many excitatory neurotransmitters including glutamate, noradrenaline and substance P (10,11).

Ha et al. have investigated the neuroprotective effect of pregabalin in spinal cord injury in rats and reported that anti-inflammatory and anti-apoptotic effects of pregabalin were demonstrated in histopathological and biochemical aspects. The authors have also suggested that pregabalin could be used as a neuroprotective agent (5,12). In our study, we have found that H-E and PAS stainings have indicated the protective effects of high dose pregabalin and also observed that the amount of erythrocyte leaking out of the vessel walls of the Bowman's capsule was higher I/R group, when compared with the controls. In the low-dose pregabalin given group, there was no change in this histopathological finding, but there was a significant improvement in 200 mg/kg pregabalin given group, suggesting a similar appearance to the control group. Likewise, although there were very slight edematous changes in I/R and low dose groups in PAS staining, there was no significant difference between control and 200 mg/kg group. There was no problem in the basal membrane in the bowel capsule inner vessels and all other tubules. Li et al. have reported that methylprednisolone treatment does not reduce caspase-3 expression after spinal cord injury (13). In another study by Ha et al., it has been mentioned that caspase-3 and p38 MAPK expressions have been decreased but Bcl-2 expression did not change in astrocyte cells, which in turn may indicate that pregabalin has anti-inflammatory and anti-apoptotic effects in neuronal tissue (5, 12).

We have found that low-dose pregabalin application reduces the expression of the p53 gene expression compared to the I/R group, whereas high-dose pregabalin does not. In our study, p53 expression was detected at different intensities in the bowel capsules, especially in the I/R group when there were no p53 expressing cells in the control group. No expression was detected in the general tubules. Interestingly, in the low-dose group, it was found that p53 expression was markedly faded but very prominent in the 200 mg/kg group.

Ha et al. have reported a lower phosphorylated MAPK expression in IR group than the pregabalin treated group and this finding supports the observed histopathological changes by taking microglia activation into account (12). At later stages, astrocytes can help regeneration through the production of various cytokines such as TGF-beta, glial cell-derived neurotrophic factor, B-FGF and VEGF. Some of these growth factors may facilitate oligodendrocyte precursor migration, proliferation and differentiation (12). In our study, VEGF was similar among the groups. Kim et al. have mentioned that intravenously administered gabapentin applied at 0.1 mg/kg, 0.5 mg/kg and 5 mg doses 20 minutes prior to occlusion has exerted a neuroprotective effect via reducing the infarct volume and brain edema in the groups and that the protective effect at 5 mg/kg dose was more prominent (14). Ha et al. have investigated the neuroprotective effects of pregabalin in spinal cord injured rats and showed that pregabalin has antiinflammatory and antiapoptotic effects via histological and biochemical parameters (5, 12).

In a study by Celik et al. which was conducted to investigate the role of pregabalin in sciatic nerve injury, it has been commented that pregabalin-treated groups had superior histopathological regeneration capacity at prominent level in peripheral nerve injury sites, when compared to the control group. In addition, they have also reported that there was an increment in sciatic functional index and TGF-beta gene expression in pregabalin groups (15). Çalikoğlu et al. have reported that pregabalin effectively prevented post-traumatic edema, inflammation and neuronal damage in their studies (16). There are studies in the literature conducted on cerebral I/R with pregabalin, peripheral neuroprotective effect of neuroprotective and sciatic nerve injury in spinal cord injury. In our study, we have also investigated the protective effects of different doses of pregabalin on renal tissue in spinal cord I/R injury. Oxidative stress occurs when the antioxidant defense system can not cope with the production of ROS. Antioxidants and other cellular redox state modulator enzyme systems are the first step in defense against ROS in all cellular and extracellular compartments. SOD, CAT and GSH-PX are the most important antioxidant enzymes against ROS.

On the other hand, lipid peroxidation markers that react with MDA and NO, such as TBARS, increase oxidative stress (17). Experimental and clinical studies have shown that antioxidants may protect from the development of I/R damage through the suppression of endogenous antioxidant enzymes and free radical production (18). Oxidative stress reactions and membrane lipid peroxidation cause oxidative damage in cerebral tissue via increasing ROS formation (19). It has been reported that endogenous antioxidant enzymes such as SOD, CAT and GSH-Px decreased after cerebral I/R injury and lipid peroxidation markers such as nitric oxide and TBARS increased in rats (17, 20).

In a study performed 24 hours after reperfusion of the middle cerebral artery occlusion, a significant decrease in CAT enzyme has been observed. In this study, it has also been indicated that CAT activities in ischemia and reperfusion groups remarkably decreased and pregabalin treatment significantly increased the activity in PI and PIR groups (18). There are several analytical methods which are utilized to measure the serum or plasma levels of many oxidants and antioxidant molecules (21). However, it is a time-consuming, expensive and difficult effort to separately measure these substances. For this reason, the "total antioxidant state" or "total oxidant state" measurement is more practical than the individual measurement of oxidant and antioxidant enzyme levels and we have preferred this way. Several methods have been used to determine oxidative stress in cerebral ischemia. There are studies in the literature reporting that TOS and OSI values increased in cerebral ischemia models (22). In another study conducted on rat brain tissues, Uzar et al. have mentioned that significant changes were found in TOS levels and OSI, while there was no change in TAS values. They have also reported that TOS levels in brain tissues of ischemic rats significantly increased when compared with the controls and that values established from the memantine group were close to the controls. Memantine has been shown to reduce the occurrence of I/R-induced damage to one point. However, memantine did not fully recover the tissue destruction (22).

In our study, we have found that low and high dose pregabalin administration reduced the I/R-induced oxidative stress injury. TOS enzyme activity was significantly higher in I/R group than in C, I/R-LP and I/R-HP groups. Similarly, TAS enzyme activity was prominently higher in I/R group than in C, I/R-LP and I/R-HP groups. Doses of single intraperitoneal (10 and 30 mg/kg) and oral (1, 3, 10 and 30 mg/kg) pregabalin in rectal hypersensitivity pain induced lipopolysaccharide-induced pain in rats suppressed rectal distention induced allodynia. In an experimental rat neuropathic pain model induced by vincristine in which pregabalin administration at 80 mg/kg dose was compared with a single intraperitoneal injection of dox-ety placebo, it has been reported that allodynia was significantly suppressed with a peak effect reached after 45 minutes of administration and lasting up to 90 minutes after the dose was given. In one study, it has been found that pregabalin (25 mg/kg i.v. and intrathecal at doses ranging from 3.8 to 60 µg) suppressed tacrolimus-dependent tactile allodynia in a photochemically induced ischemic nerve lesion (23).

CONCLUSION

Pregabalin treatment in spinal cord ischemia reperfusion injured rats increased TAS and TOS activity in renal tissues. Taken together, data established from the current study suggest that pregabalin affects different mechanisms in I/R injury and that different parameters should be evaluated to get more insight into the underlying mechanisms in protective effect of pregabalin on I/R-induced renal tissue injury. According to these findings, pregabalin may be useful for the prevention of ischemia reperfusion in spinal cord injury.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Kazanci B, Ozdogan S, Kahveci R, Gokce EC, Yigitkanli K, Gokce A, et al. Neuroprotective Effects of Pregabalin Against Spinal Cord Ischemia-Reperfusion Injury in Rats. *Turk Neurosurg* 2017; 27:952-61.
2. Nigwekar SU, Kandula P, Hix JK, Thakar CV. Off-pump coronary artery bypass surgery and acute kidney injury: a meta-analysis of randomized and observational studies. *Am J Kidney Dis* 2009; 54: 413-23.
3. Homer-Vanniasinkam S, Crinnion JN, Gough MJ. Post-ischaemic organ dysfunction: a review. *Eur J Vasc Endovasc Surg* 1997; 14: 195-203.
4. Martin L, Rabasseda X, Leeson P, Castaner J. Pregabalin. *Drugs of the Future* 1999; 24: 862-70.
5. Ha K, Kim Y, Rhyu K, Kwon S. Pregabalin as a neuroprotector after spinal cord injury in rats. *Eur J Spine* 2008; 17: 864-72.
6. Jiang G, Liul X, Wang M, Chen H, Chen Z, Qiu T. Oxymatrine ameliorates renal ischemia-reperfusion injury from oxidative stress through Nrf2/HO-1 pathway. *Acta Cir Bras* 2015; 30: 422-9.
7. Khajuria A, Tay C, Shi J, Zhao H, Ma D. Anesthetics attenuate ischemia reperfusion induced renal injury: Effects and mechanisms. *Acta Anaesthesiol Taiwan* 2014; 52: 176-84.
8. Wan X, Hou LJ, Zhang LY, Huang WJ, Liu L, Zhang Q, et al. IKKα is involved in kidney recovery and regeneration of acute ischemia/reperfusion injury in mice through IL-10-producing regulatory T cells. *Dis Model Mech* 2015; 8: 733-42.
9. Kirişçi İ, Okutan H, Savaş Ç, Yönden Z, Delibaş N. Deneysel Aortik İskemi-Reperfüzyon modelinde renal hasara gadolinium klorürün etkisi. *Turkish J Vasc Surg* 2005; 14: 13-8.
10. Brodie MJ. Pregabalin as adjunctive therapy for partial seizures. *Epilepsia* 2004; 45(Suppl 6): 19-27.
11. Partridge BJ, Chaplan SR, Sakamoto E, Yaksh TL. Characterization of the effects of gabapentin and 3-isobutyl-aminobutyric acid on substance P-induced thermal hyperalgesia. *Anesthesiology* 1998; 88: 196-205.
12. Ha KY, Carragee E, Cheng I, Kwon SE, Kim YH. Pregabalin as a Neuroprotector after Spinal Cord Injury in Rats: Biochemical Analysis and Effect on Glial Cells. *J Korean Med Sci* 2011; 26: 404-11.
13. Li M, Ona VO, Chen M, Kaul M, Tenneti L, Zhang X, et al. Functional role and therapeutic implications of neuronal caspase-1 and -3 in a mouse model of traumatic spinal cord injury. *Neuroscience* 2000; 99: 333-42.
14. Kim YK, Leem JG, Sim JY, Jeong SM, Joung KW. The effects of gabapentin pretreatment on brain injury induced by focal cerebral ischemia/reperfusion in the rat. *Korean J Anesthesiol* 2010; 58: 184-90.
15. Celik M, Kose A, Kose D, Karakus E, Akpınar E, Calik M, et al. The double edge sword: effects of pregabalin on experimentally induced sciatic nerve transection and crush injury in rats. *Int J Neurosci* 2015; 125(11): 845-54.

16. Calikoglu C, Aytekin H, Akgul O, Akgul MH, Gezen AF, Akyuz F, et al. Effect of Pregabalin in Preventing Secondary Damage in Traumatic Brain Injury: An Experimental Study. *Med Sci Monit* 2015; 21:813-20.
17. Wu JQ, Kosten TR, Zhang XY. Free radicals, antioxidant defense systems, and schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2013;46:200-6.
18. Aşçı S, Demirci S, Aşçı H, Doğuç DK, Onaran İ. Neuroprotective Effects of Pregabalin on Cerebral Ischemia and Reperfusion. *Balkan Med J* 2016; 33:221-7.
19. Reddy VD, Padmavathi P, Kavitha G, Saradamma B, Varadacharyulu N. Alcohol-induced oxidative/nitrosative stress alters brain mitochondrial membrane properties. *Mol Cell Biochem* 2013;375:39-47.
20. Ozerol E, Bilgic S, Iraz M, Cigli A, Ilhan A, Akyol O. The protective effect of erdosteine on short-term global brain ischemia/ reperfusion injury in rats. *Prog Neuropsychopharmacol Biol Psychiatry* 2009;33:20-4.
21. Tarpey MM, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. *Am J Physiol Regul Integr Comp Physiol* 2004; 286:R431-44.
22. Uzar E, Acar A, Fırat U, Evliyaoğlu O, Alp H, Tüfek A, et al. Protective Effect of Caffeic Acid Phenethyl Ester in Rat Cerebral Ischemia/Reperfusion Damage. *Turk Norol Derg* 2011;17:131-6.
23. Joyce M, Kelly C, Winter D, Chen G, Leahy A, BouchierHayes D. Pravastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, attenuates renal injury in an experimental model of ischemia-reperfusion. *J Surg Res* 2001; 101:79-84