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Original Article

The effect of tramadol on oxidative stress total antioxidant levels in rats with renal ischemia-reperfusion injury

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ABSTRACT

Objective: To evaluate the protective effect of tramadol on renal tissue in rats with induced renal ischemia-reperfusion injury (I/R injury), and its effects on oxidative stress.

Material and methods: Thirty adult, male Wistar rats weighing 250–300 g were selected as subjects. Rats were randomized into 3 groups: group 1, sham; group 2, renal I/R injury; and group 3, renal I/R+Tramadol. In order to obtain ischemia in groups 2 and 3, renal artery was clamped for 1 h. Total oxidant status (TOS) and total antioxidant capacity (TAC) were analyzed using biochemical assays in the serum samples.

Results: TOS values were measured as 1.68±0.4 in group 1, 3.35±1.0 in group 2, and 3.49±0.9 in group 3. When group 1 was compared with group 2 and group 3, the TOS values of group 1 were significantly lower ($p<0.05$), whereas there was no difference between group 2 and group 3 ($p>0.05$). TAC values were measured as 1.65±1.4 in group 1, 1.85±0.1 in group 2, and 2.79±0.6 in group 3. The antioxidant status of group 1 was not significantly different from that of group 2 ($p>0.05$), whereas there was a significant difference between group 1 and group 3 ($p>0.05$).

Conclusions: Tramadol has positive effects on antioxidant levels in renal I/R injury. We think that tramadol may be used in patients who underwent renal surgery and have I/R injury risk. There is a need for studies on this subject including human series.

Keywords: Antioxidants; ischemia; reperfusion injury; tramadol.

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Introduction

Complete loss or decrease of blood supply in a tissue may lead to ischemia. The restoration of blood flow in an ischemic tissue is referred to as reperfusion. Free oxygen radicals and inflammatory cells especially begin to increase in the tissue with reperfusion. This increase causes reperfusion injury in addition to ischemic injury.[1,2]

Acute renal failure (ARF) is a common disease with high morbidity and mortality. Current treatment options for ARF are limited, and the mortality rate ranges between 30% and 50%.[3] Renal ischemia-reperfusion injury (I/R injury) is one of the most common causes of ARF. I/R injury is characterized by changes in cell metabolism, apoptosis, free radical formation, and inflammation, resulting in renal tubular cells becoming detached from the basement membrane and spilling into the urine.[4-6] The severity of the I/R injury depends on the duration of ischemia and the sufficiency of collateral circulation.[7]

Tramadol hydrochloride is used as an effective analgesic for acute and chronic pain for cases such as cancer and neuropathic and postoperative pain. It has a low affinity to the M-opioid receptor and activates descending inhibitory systems by inhibiting the reuptake of monoamines in the central nervous system.[8,9] Considering the importance of renal functions, preventing the formation of injury decreases both the mortality and morbidity rates. Therefore, studies were conducted on the use of various substances that have antioxidant and/or anti-inflammatory properties in order to minimize

the above-mentioned injury. Recently, there have been reports indicating that tramadol, which is an opioid, prevents I/R injury in brain, myocardial, and muscle tissue.[10-12]

In the current study, we aimed to evaluate the protective effect of tramadol on renal tissue in rats with induced renal I/R injury, and its effects on oxidative stress.

Material and methods

Rats used in this study were obtained from the experimental animal research center of Gaziantep University. The approval for the experimental protocol and animal care methods were received from the experimental animal research committee of the center (Ethics Committee: 15.03.2017/25).

Thirty adult, male Wistar rats weighing 250–300 g were selected as subjects. Rats were kept and cared for in an environment that has a ventilation system that provides a room temperature of 21±2°C, relative humidity between 40% and 60%, cage illuminance of 40 lux, light period of 12 h of light/dark, and air change of 16/h during the experiments. All rats in the control and experimental groups had free access to food and water (*ad libitum*).

Experimental groups

Rats were randomized into 3 groups. There were 10 subjects in each group. All rats were anesthetized by 50 mg/kg intraperitoneal ketamine and 10 mg/kg xylazine hydrochloride. After the anesthesia, rats were fixed on the surgical table in a supine position using adhesive tape. The surgical site was shaved, and asepsis was provided by povidone-iodine.

Group 1 (Sham, n=10): Abdominal dissection was performed 30 min after anesthesia; the abdomen was incised and kept for 1 h, and then the abdomen layers were closed.

Group 2 (I/R, n=10): Abdominal dissection was performed 30 min after anesthesia. The left renal artery was isolated by abdominal incision, and renal ischemia was provided for 60 min using a non-traumatic clamp. After the ischemia period, the vascular clamp was removed, and the incision line was sutured using 3/0 polypropylene sutures. After renal ischemia for 60 min, I/R injury was analyzed after 24 h.

Main Points:

- Tramadol can be used as a preventive agent for renal reperfusion ischemia injury.
- Tramadol is effective on total antioxidant levels in urine after ischemia.
- This agent may be a new application, considering that it will reduce kidney damage, especially in kidney surgeries.

Group 3 (I/R+Tramadol, n=10): Abdominal dissection was performed 30 min after anesthesia. Renal ischemia was provided 30 min after administration of 40 mg/kg tramadol hydrochloride. After the ischemia period, the vascular clamp was removed, and the incision line was sutured using 3/0 polypropylene sutures. After renal ischemia for 60 min, I/R injury was analyzed after 24 h.

All groups were administered 2 mL 0.09% NaCl intraperitoneally in order to prevent dehydration throughout the abdominal dissection. In addition, the incision site was covered by a wet sterile sponge. Homeothermic plate with a rectal probe (ATC 2000, WPI, Sarasota, FL, USA) was used during the operation in order to prevent hypothermia.

Ischemia-reperfusion monitoring

In order to obtain ischemia in group 2 and group 3, the renal artery was clamped for 1 h. In the meantime, complete loss of vascular circulation in the renal tissue was confirmed using Laser Doppler Flowmetry (LDF) (Periflux System 5000, Perimed, Sweden). After the ischemia period, the artery was unclamped, and the achievement of reperfusion was shown by LDF. LDF measurements that showed ischemia and reperfusion formation were recorded and shown in Figure 1.

Biochemical analysis

Rat serum samples were prepared for biochemical assays by centrifuging for 10 min at 3000 rpm. All serum samples were kept at –80°C until the assays were performed.

Total oxidant status (TOS) assay

TOS assays in rat serum were performed using kits (Rel Assay Diagnostics Total Oxidant Status Assay Kit) that were previously developed by Erel[13] with an automated analyzer (Tokyo Boeki, Prestige 24, Tokyo, Japan). This method is a colorimetric method based on the cumulative oxidation of ferrous ion to ferric ion by oxidant molecules present in the samples that will undergo oxidant capacity assay. H_2O_2 was used as a standard, and the results were calculated as μmol H_2O_2 Eq/L.

Total antioxidant capacity (TAC) assay

TAC assays in rat serum were performed using kits (Rel Assay Diagnostics Total Antioxidant Status 39 Assay Kit) that were previously developed by Erel[14] with an automated analyzer (Tokyo Boeki, Prestige 24, Tokyo, Japan). This method measures the TAC of the samples against strong reactive oxygen species (mmol Trolox Eq/L).

Statistical analysis

A power analysis was performed in order to determine the number of rats that will be used in the study, and the number was determined as 7. A Windows-compatible IBM Statistical Package for the Social Sciences 20.0 (IBM SPSS Corp.; Armonk,

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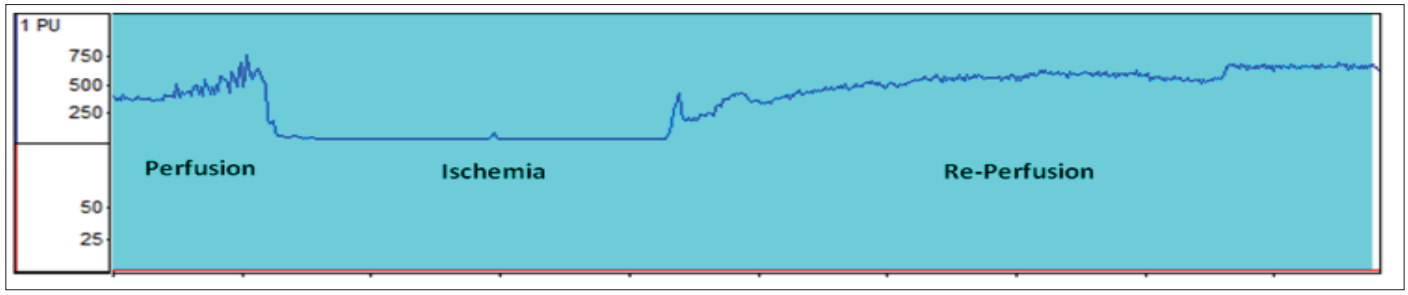

Figure 1. Real-time monitorization of microvascular perfusion with LDF in renal tissue that was rendered ischemic

Table 1. TOS and TAC values of groups

	Group 1	Group 2	Group 3	p
TOS (Mean±SD) μmol H_2O_2 Eq./L	1.68±0.4	3.35±1.0	3.49±0.9	<0.05
TAC (Mean±SD) mmol Trolox Eq./L	1.65±1.4	1.85±0.1	2.79±0.6	<0.05

TOS: total oxidant status; SD: standard deviation; TAC: total antioxidant capacity

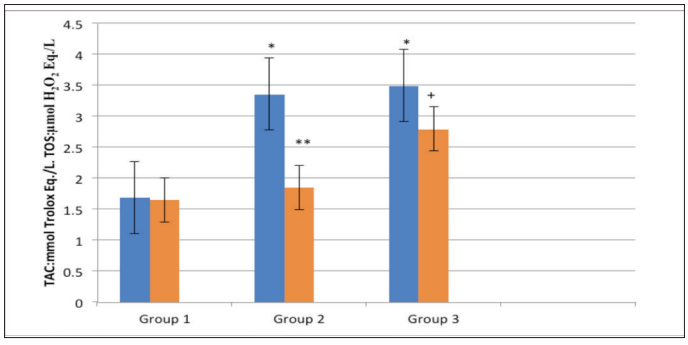

Figure 2. Serum total antioxidant capacity (TAC), and total oxidant status (TOS) assays (mean±standard deviation)
*Group 1 significant difference with respect to TOS (p<0.05)
**Group 1 significant difference with respect to TAC (p>0.05)
+Group 2 significant difference with respect to TAC (p<0.05)

NY, USA) package program was used for the statistical analysis. The data were evaluated with Kruskal–Wallis H. All data were calculated as mean±standard deviation. A p-value of <0.05 was considered significant.

Results

TOS (mean±standard deviation) values were measured as 1.68±0.4 in group 1, 3.35±1.0 in group 2, and 3.49±0.9 in group 3. When group 1 was compared with group 2 and group 3, the TOS values of group 1 were significantly lower (p<0.05), whereas there was no difference between group 2 and group 3 (p>0.05). TAC values (mean±standard deviation) were measured as 1.65±1.4 in group 1, 1.85±0.1 in group 2, and 2.79±0.6 in group 3 (Table 1). The antioxidant status of group 1 was not significantly different from that of group 2 (p>0.05), whereas there was a significant difference between group 1 and group 3 (p>0.05). In addition, group 3 had significantly higher antioxidant status than group 1 and group 2 (p<0.05) (Figure 2).

None of the groups exhibited major or minor surgical complications associated with the operations; however, 1 rat died in group 3.

Discussion

The basis of the physiopathology of I/R injury is the acute inflammatory response. Organ dysfunction and organ failure may stem from complex mechanisms such as free oxygen radicals and leukocyte aggregation.[15-17] In previous studies, various therapeutic approaches such as hypothermia, antioxidant substances, and hypertonic saline solutions have been used to minimize the damage caused by oxidative stress on the physiology of the body under I/R.[18-21] Tramadol hydrochloride is used as an effective analgesic for acute and chronic pain for cases such as cancer and neuropathic and postoperative pain. It has a low affinity to the M-opioid receptor and activates descending inhibitory systems by inhibiting the reuptake of monoamines in the central nervous system.[9,22]

Recent research indicated that tramadol had antioxidant effects by reducing lipid peroxidation, and it could be used for the treatment of brain and lung injuries after ischemia-reperfusion of the skeletal muscle.[10,11] In addition, Takhtfooladi et al.[12] investigated the effect of tramadol on the myocardium by inducing I/R injury in a rat model. The authors reported that superoxide dismutase, catalase, and glutathione peroxidase levels were higher than those in the sham group. Also, tissue malondialdehyde (MDA) levels were significantly increased in the I/R injury group, and this increase was not observed in the tramadol group.

In the histopathological examination, microscopic hematuria, edema, neutrophil infiltration, and necrosis were evaluated, and tramadol group had significantly fewer changes compared with the I/R injury group. Consequently, the authors emphasized that treatment with tramadol alleviated myocardial injuries caused by skeletal muscle I/R.

A study that investigated the effect of tramadol on hepatic tissue was reported by Mahmoud et al.[23] The biochemical and histopathological analyses were performed after the I/R injury, after the administration of tramadol before clamping the hepatic vascular structures. According to the study, the tramadol group had a mild injury due to reducing apoptotic cell death and structural changes. In addition, it was also found that the decreased levels of inflammation markers such as tumor necrosis factor-alpha (TNF-alpha), TNF-alpha/interleukin-10 (IL-10) ratio, and nuclear factor-Kappa B gene expression were associated with tramadol. The drug was also found to increase anti-inflammatory cytokine and IL-10 levels in hepatic tissues. Furthermore, tramadol reduced oxidative stress parameters other than manganese superoxide dismutase activity. Eventually, it was reported that tramadol had hepatoprotective effects against hepatic I/R injury, due to its anti-inflammatory, antiapoptotic, and antioxidant effects.

In another study evaluating the effects of tramadol on rat skeletal muscle after I/R injury, PO_2 and HCO_3 levels were found to be higher in I/R injury+tramadol group than in I/R injury group. Moreover, serum and tissue MDA levels were significantly higher in the group that received tramadol. In addition, muscle tissue glutathione, superoxide dismutase, and catalase levels were significantly lower in the I/R group than in sham and tramadol groups. From the histopathological aspect, muscle changes were less commonly seen in the tramadol group. As a result of the studies, from the histological, histochemical, and serum biochemical perspectives, treatment with tramadol alleviated metabolic injuries in skeletal muscle ischemia and reperfusion in this experimental model.[24]

The effects of tramadol, which were experienced in many studies as mentioned above, were formed on the basis of our study. In the current study, the beneficial effect of tramadol in rats on reperfusion after ischemia could not be shown histopathologically. Oliveira et al.[25] evaluated the effects of remote ischemic preconditioning and tramadol on 25 rats with induced I/R injury. They measured the levels of MDA, which is a lipid peroxidation product in oxidative stress response, in the plasma and renal tissue. The authors reported that remote ischemic preconditioning was more effective in reducing renal I/R injury than the administration of tramadol or association of both treatments.

In our study, the effects of tramadol on oxidative stress, and antioxidant levels were measured in rats, and it was seen that tramadol significantly increased total antioxidant level, similar to previous studies. Therefore, it was concluded that tramadol was an effective agent in reducing reactive oxygen species after I/R injury. In addition, TAC levels were similar in rats that did not have I/R injury (group 1), and it could be interpreted that tramadol preserved the antioxidant level. However, considering the TOS level, it was found that tramadol did not provide any advantages, and TOS values were similar to the group with induced I/R injury (group 2). As a result of the biochemical assays, it can be concluded that tramadol increases oxidative stress by increasing the TAC level, and thus decreases renal injury.

The limitation of this study was the lack of groups that received long-term administration of tramadol, and the groups had different recovery periods. In accordance with the obtained data, tramadol has positive effects on the antioxidant level in I/R injury. With long-term studies in this area, we think that tramadol may be used in patients who underwent renal surgery and a risk of I/R injury. There is a need for studies on this subject including human series.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (15.03.2017/25).

Informed Consent: N/A.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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