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EXPERIMENTAL STUDY

Effect of different doses of pregabalin on skeletal muscle ischaemia-reperfusion injury in rats

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ABSTRACT

AIM/INTRODUCTION: Analgesic, anti-inflammatory and anti-apoptotic effects of pregabalin have been shown previously. In this study, we investigated the protective effect of different doses of pregabalin on skeletal muscle IR injury in rats.

MATERIALS AND METHODS: 24 rats were randomly divided into 4 groups (Control, Ischaemia-Reperfusion (IR), IR-Pregabalin 50 mg, IR-Pregabalin 200 mg). Following IR, serum Ischemia Modified Albumin (IMA) and tissue Paraoxonase (PON) were studied and gastrocnemius muscle tissue was removed for histopathologic examination.

RESULTS: Interstitial inflammation was higher in the IR group than in the control and Pregabalin 200 mg groups ($p = 0.037$, $p = 0.037$, respectively). Congestion was higher in the IR group than in the control, Pregabalin 50 and 200 mg groups ($p = 0.001$, $p = 0.004$, $p = 0.004$, respectively). PON was lower in the IR group than in the Control, Pregabalin 50 and 200 mg groups ($p = 0.001$, $p = 0.007$, $p = 0.015$, respectively). IMA was higher in the IR group than in the Control, Pregabalin 50 and 200 mg groups ($p < 0.0001$, all).

CONCLUSION: We think that administration of pregabalin, more prominent at 200 mg, can reverse the injury that occurs in the skeletal muscle of IR-induced rats. Pregabalin can be safely used for analgesia in cases of IR *(Tab. 2, Fig. 9, Ref. 41)*. Text in PDF *www.elis.sk.*

KEY WORDS: hind limb ischemia reperfusion, pregabalin, histopathology, IMA, PON.

Introduction

Cellular damage after reperfusion of previously viable ischemic tissues in lower extremity is a common and critical clinical incident. If blood flow is re-established after reperfusion, substances produced as a result of metabolites' oxidation are spread throughout the body by systemic circulation. Oxygen free radicals are the most important and toxic substance produced in various clinical conditions (1). What all of these clinical situations have in common is either hypoxic microenvironments followed by reoxygenation or ischaemic microenvironments followed by reperfusion. As reperfusion progresses, generally systemic inflammatory response syndrome and multiple organ failure (kidney, respiratory and circulatory system, etc.) follow local edema and muscle necrosis (2–6).

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Pregabalin is a structural analog of gamma-aminobutyric acid (GABA) (7). Currently, pregabalin is indicated for the management of neuropathic pain associated with diabetic peripheral neuropathy (8, 9), postherpetic neuralgia (10, 11), and management of fibromyalgia (12, 13). In addition, pregabalin is used frequently in the treatment of anxiety (14). It has a distinct mechanism of action relative to other anti-anxiety agents ($\alpha_2\delta$ binding at presynaptic voltage dependent calcium channels leading to inhibition of excitatory neurotransmission). Pregabalin does not interact with liver enzymes and 95 % of it is excreted by kidneys. Besides these clinical indications, it has some new properties that we need to focus on. Pregabalin has been used to protect against I/R injury in many organs (15, 16).

Benefits of pregabalin use to prevent local and distal tissue injury due to I/R has been well documented so far. However, not much is known about the protective effect of low and high doses of pregabalin on I/R injury. The primary aim of this study was to investigate the effect of low and high doses of pregabalin on skeletal muscle I/R injury in rats.

Materials and methods

Animals and experimental protocol

This study was carried out in Gazi University Physiology Laboratory with the approval of the Ethics Committee of Experimental Animals of our university. All of the procedures were performed

according to accepted standards of Guide for the Care and Use of Laboratory Animals.

In our study, 24 Wistar Albino rats weighing between 250 and 300 g, raised under the same environmental conditions, were used. The rats were kept under 20–21 °C at cycles of 12-hour daylight and 12-hour darkness and had free access to food until 2 hours before the anesthesia procedure. The animals were randomly separated into four groups, each containing 6 rats. Midline laparatomy was done under general anesthesia.

Control group

A midline laparotomy was performed without any extra surgical intervention. After 2 hours of follow-up blood and tissue samples were collected and animals were then sacrificed.

I/R group

Midline laparotomy was performed in the same way. Infrarenal aorta was clamped for 2 hours. The clamp was removed and then reperfusion was started. Reperfusion lasted for two more hours. In the end, after blood and tissue sampling, the rats were sacrificed.

I/R group with pregabalin 50 mg

Similiar steps were followed as mentioned above but in addition to the procedure pregabalin was given (50 $mg.kg^{-1}$) intraperitoneally for 30 minutes before ischemia period. After collecting blood and tissue samples, rats were sacrificed at the end of two-hour reperfusion period.

I/R group with pregabalin 200 mg

Similiar steps were followed as mentioned above but in addition to the procedure, pregabalin was given (200 $mg.kg^{-1}$) intraperitoneally for 30 minutes before ischemia period. After collecting blood and tissue samples, rats were sacrificed at the end of two-hour reperfusion period.

Rats were anesthetized with ketamine (100 $mg.kg^{-1}$, intraperitoneally) and intracardiac blood samples and tissue samples were collected.

A total of 25 muscle tissues including control and experimental cases were processed for paraffin sections. Formalin fixation, dehydration, clearing with xylene, paraffin wax infiltration and blocking steps were performed, respectively. Sections of four-micron thickness were taken from paraffin blocks. The presence of interstitial inflammation, edema, congestion, atrophy, necrosis and increased fat tissue are evaluated according to hematoxylin-eosin sections. The presence of fibrosis was evaluated according to Masson's Trichrome staining.

Paraoxonase activity assay

Paraoxonase activity was determined spectrophotometrically at 25 °C with paraoxon (diethyl p-nitrophenyl phosphate) (1 mM) in 50 mM glycine/NaOH (pH 10.5) containing 1 mM $CaCl_2$. The enzyme assay was based on the estimation of p-nitrophenol at 412 nm. The molar extinction coefficient of p-nitrophenol (e = 18,290 M^{-1} cm^{-1} at pH 10.5) was used to calculate enzyme activity (17). One enzyme unit was defined as the amount of enzyme that catalyzes the hydrolysis of 1 μmol of substrate at 25 °C (18).

Ischemia-modified albumin Asssay

Ischemia-modified albumin (IMA) was determined by a manual colorimetric assay described by Bar−Or et al (19) and called the Co(II)−albumin binding assay. This method consists of adding a known amount of exogenous Co(II) to a plasma sample and measuring unbound Co(II) colorimetrically using dithiothreitol (DTT). The results are given in absorbance units (ABSU).

Results

The histopathological parameters of interstitial inflammation, atrophy, increase in fat tissue, and congestion were significantly different between the groups ($p = 0.040$, $p = 0.001$, $p = 0.023$, $p = 0.003$, respectively). Interstitial inflammation was significantly higher in the IR group than in the control and Pregabalin 200 mg groups ($p = 0.037$, $p = 0.037$, respectively). Congestion was significantly higher in the IR group than in the control, Pregabalin 50 and 200 mg groups ($p = 0.001$, $p = 0.004$, $p = 0.004$, respectively). Atrophy was higher in all groups compared to the control group ($p = 0.001$, $p < 0.0001$, $p = 0.003$, respectively). Fat tissue increase was significantly higher in the IR group compared to the control group ($p = 0.003$) (Tab. 1, Figs 1–5). Collagen increase was detected with Masson trichrome stain (Figs 6–9).

PON was significantly lower in the IR group than in the Control, Pregabalin 50 and 200 mg groups ($p = 0.001$, $p = 0.007$, $p = 0.015$, respectively). PON was found similar among the other groups (Tab. 2).

IMA was significantly higher in the IR group than in the Control, Pregabalin 50 and 200 mg groups ($p < 0.0001$, all). IMA was found similar among the other groups (Tab. 2).

Tab. 1. Rat muscle tissue histopathological findings (Mean±SD).

	Group C (n=6)	Group IR (n=6)	Group IR-P50 (n=6)	Group IR-P200 (n=6)	p **
Interstitial edema	0.00±0.00*	0.50±0.22	0.33±0.22	0.00±0.00	0.040
Interstitial inflammation	0.00±0.00	0.33±0.17	0.17±0.17	0.17±0.17	0.540
Fibrosis	0.00±0.00	0.33±0.17	0.17±0.17	0.00±0.00	0.269
Necrosis	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	–
Atrophy	0.00±0.00	1.00±0.26&	1.17±0.17&	0.83±0.17&	0.001
Fat tissue increase	0.00±0.00*	0.83±0.17	0.50±0.22	0.33±0.21	0.023
Congestion	0.00±0.00*	0.83±0.17	0.17±0.17*	0.17±0.17*	0.003

p **: Kruskal–Wallis test significance level $p < 0.05$, & $p < 0.05$: Compared with Control group; * $p < 0.05$: Compared with group IR

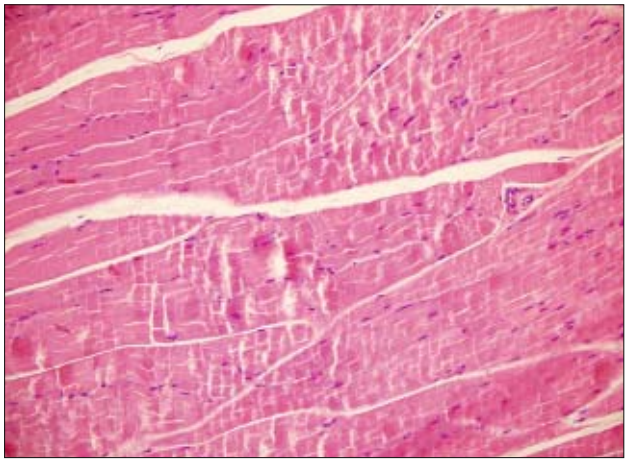

Fig. 1. Control Group normal muscle tissue (H&Ex200).

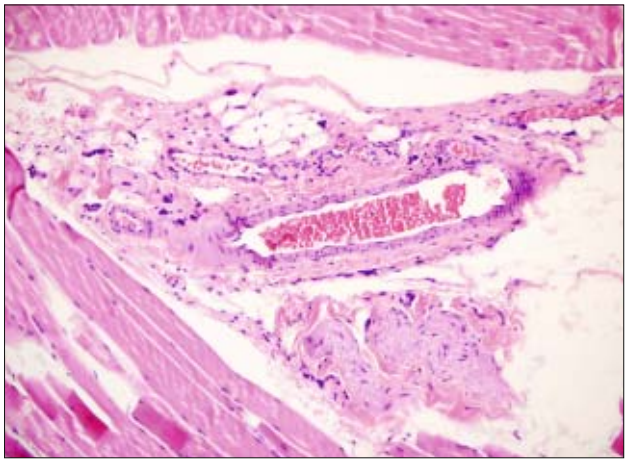

Fig. 2. Ischemia-reperfusion Group muscle tissue: atrophy, increase in fat tissue, congestion (H&Ex200).

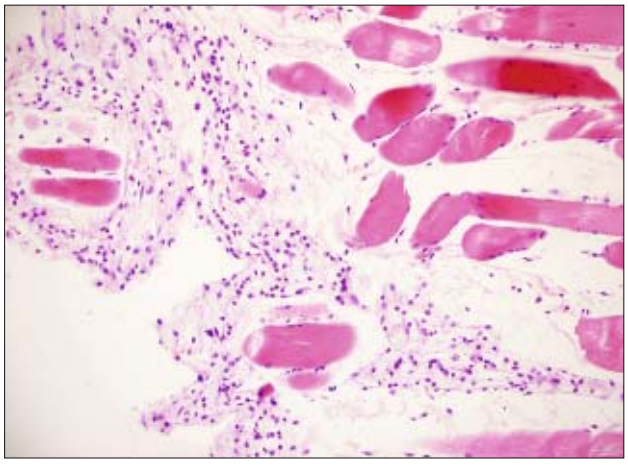

Fig. 3. Ischemia-reperfusion Group muscle tissue: atrophy, interstitial edema, inflammation (H&Ex200).

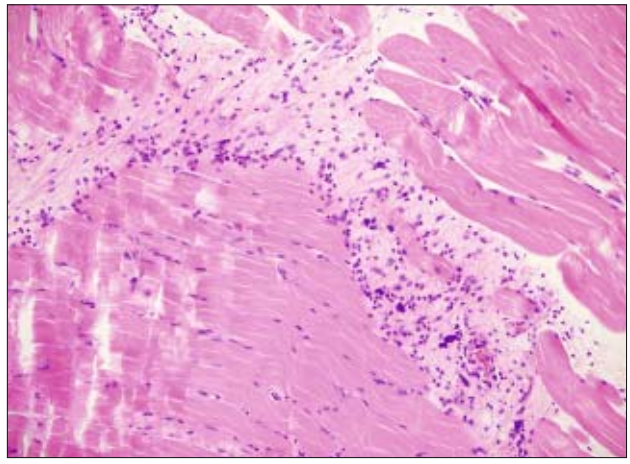

Fig. 4. Ischemia-reperfusion Pregabalin 50 mg Group muscle tissue: atrophy, interstitial edema, inflammation (H&Ex200).

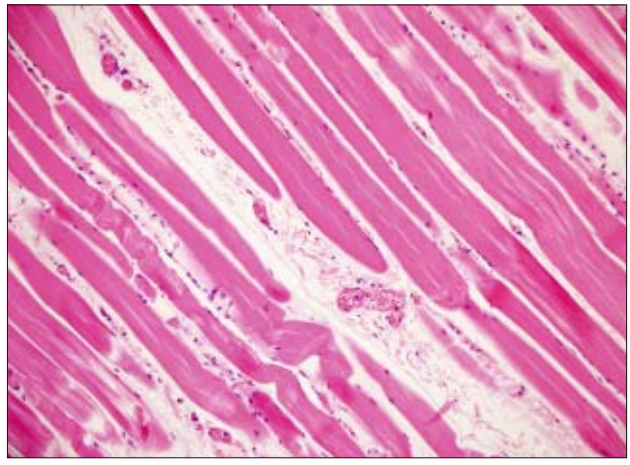

Fig. 5. Ischemia-reperfusion Pregabalin 200 mg Group muscle tissue: minimal congestion, edema (H&Ex200).

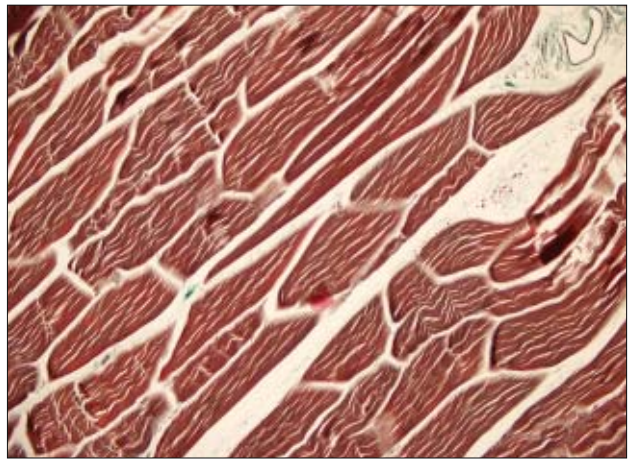

Fig. 6. Control Group normal muscle tissue (Masson Trichrome X200).

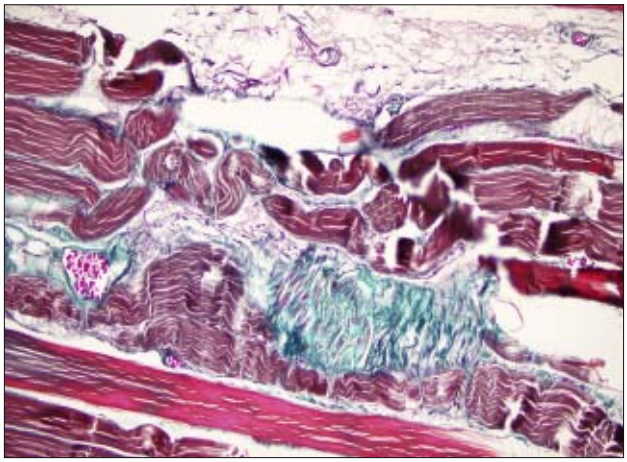

Fig. 7. Ischemia-reperfusion Group muscle tissue: fibrosis and fatty tissue increase (Masson Trichromex200). (Muscle fibers red brown, fibrosis-connective tissue increase = collagen increase green).

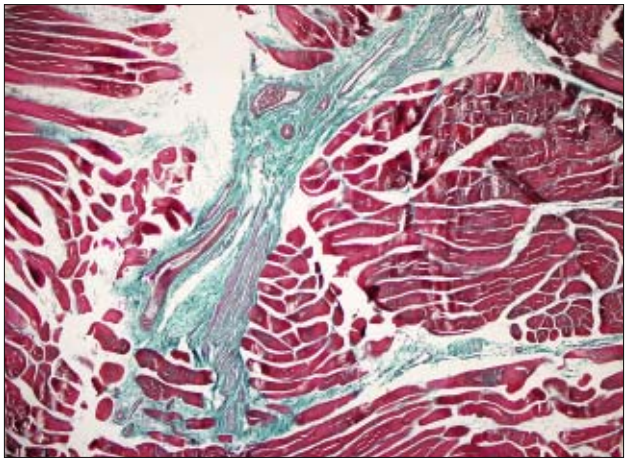

Fig. 8. Ischemia-reperfusion Pregabalin 50 mg Group muscle tissue: fibrosis (Masson Trichromex200).

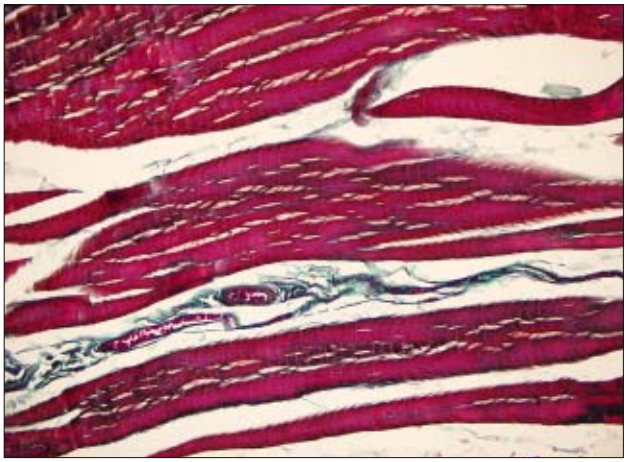

Fig. 9. Ischemia-reperfusion Pregabalin 200 mg Group muscle tissue (Masson Trichromex200).

Discussion

Ischemia followed by reperfusion in skeletal muscle causes an important clinical problem. Reperfusion followed by recanalization of the occluded blood vessels may also contribute to skeletal muscle injury. Free oxygen radicals released out after I/R injury have a remarkable mediator role in several organs' I/R injuries (20–22). Recently, various approaches for protection from I/R injury were supported with the help of various antioxidant molecules (20, 23–25).

Pregabalin is a structural analog of gamma-aminobutyric acid and has analgesic, anticonvulsant, anxiolytic and opioid-sparing effects. Pregabalin is a gabapentin derivative and has superior pharmacokinetic activity although it shows a similar effect (26). In animal studies, pregabalin, such as gabapentin, has also been shown to be effective in several models of neuropathic pain, incisional and inflammatory injury (27). There are studies showing that preoperative administration of pregabalin reduces acute postoperative pain, analgesic consumption and incidence of chronic neuropathic pain (28, 29).

In studies of Pregabalin, different methods of application have been used so far and there is no standard dose agreed for I/R injury treatment. Therefore, in our study we aimed to detect the protective effect of low and high doses of pregabalin on skeletal muscle ischemia-reperfusion injury in rats.

PON enzyme activity is critical for the physiological function of several key metabolic pathways, and plasma level of it is related with the pathogenesis of many diseases. In many clinical research studies, these findings have been reported (30–32). Oxidative stress especially appears in reperfusion after ischemia. Antioxidant defense system has a significant role in preventing the oxidative stress damage (20, 25, 33, 34). PON enzyme reduces oxidant activity to protect LDL from oxidation damage induced by free radical (35–37). In our study, PON was significantly lower in the IR group than in the Control, Pregabalin 50 and 200 mg groups. However, PON level was similar among the other groups. Pregabalin increased PON enzyme activity.

Serum IMA levels may be a beneficial non-specific tissue ischemia biomarker (38). Active oxygen forms, particularly hydroxyl

Tab. 2. PON and IMA findings (Mean±SD).

	Group C (n=6)	Group IR (n=6)	Group IR-P50 (n=6)	Group IR-P200 (n=6)	p **
PON (IU/mg.protein)	145.34±17.34*	58.22±3.61	129.28±16.78*	122.94±13.28*	0.001
IMA (ΔABSU.un)	0.29±0.04*	0.62±0.05	0.36±0.04*	0.35±0.04*	<0.0001

p **: Kruskal–Wallis test significance level $p < 0.05$, * $p < 0.05$: Compared with group IR

radicals, might modify chemically human albumin and produce IMA (39). An increase in protein damage manifests as increased serum levels of IMA. It has been shown previously that IMA level estimated in blood serum sample has a prognostic value for acute ischemic stroke (40). Also, it is reported that IMA level increases in early ischemia and remains high (41). In our study, IMA was significantly higher in the IR group than in the Control, Pregabalin 50 and 200 mg groups. However, IMA was similar among the other groups.

The histopathological examination found interstitial inflammation to be significantly higher in the IR group than in the control and Pregabalin 200 mg groups; congestion was significantly higher in the IR group than in the control and Pregabalin 200 mg groups. Also, atrophy was higher in all groups compared to the control group and fat tissue increase was significantly higher in the IR group compared to the control group. In addition, collagen increase was detected with Masson trichrome stain. These results indicate that pregabalin has a protective effect on skeletal muscle damage created with IR.

In conclusion, we found out that pregabalin increased the antioxidant ability and has a protective effect on skeletal muscle IR injury in rats. Also, evaluation of serum PON, IMA levels and histopathological findings of skeletal muscle tissue showed that pregabalin had valuable protective effects on skeletal muscle I/R injury; hence pregabalin can be acknowledged as a protective theraphy for skeletal muscle I/R injury. We think that administration of pregabalin, more prominent at 200 mg, can reverse the injury that occurs in the skeletal muscle of IR-induced rats. Pregabalin can be safely used for analgesia in cases of IR. To the best of our knowledge there is no preclinical study comparing the effect of different doses of pregabalin on skeletal muscle I/R Injury. As we could not investigate the long-term results of pregabalin treatment of skeletal muscle IR injury, we still think that these promising results should further be supported by more detailed studies with larger volumes.

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