

# The Effect of Oral Melatonin Premedication on Pneumatic Tourniquet Induced Ischemia Reperfusion Injury in Lower Extremity Operations

Tuba Berra Saritas<sup>1\*</sup>, Sadik Buyukbas<sup>2</sup>, Kevser Peker<sup>3</sup>, Hale Borazan<sup>1</sup>, Kemal Basarali<sup>4</sup>, Ibrahim Tuncay<sup>5</sup>, Alper Kiliçarslan<sup>1</sup> and Seref Otelcioglu<sup>1</sup>

<sup>1</sup>Department of Anesthesiology and Reanimation, Meram Medical School, Necmettin Erbakan University, Konya, Turkey

<sup>2</sup>Department of Biochemistry, Meram Medical School, Necmettin Erbakan University Konya, Turkey

<sup>3</sup>Kırşehir Ahi Evran University, Government Hospital, Kırşehir, Turkey

<sup>4</sup>Dicle University Medical School, Diyarbakır Turkey

<sup>5</sup>Department of Orthopaedics, Bezmi Alem University, Istanbul, Turkey

## Abstract

**Purpose:** Melatonin is a well-known antioxidant molecule and protector against ischemia reperfusion (I/R) injury. It has a tissue protective effect against I/R injury. **Methods:** We aimed to evaluate the effect of oral melatonin, on I/R injury in patients undergoing arthroscopic knee surgery under spinal anaesthesia.

**Methods:** Malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), haemodynamics, respiration rate, atropine and efedrin need, sedation scores were all measured for this purpose.

**New Results:** There were no significant differences between groups in terms of age, weight, height, sex, ASA classification, BMI, tourniquet time and operation time. Plasma concentrations of MDA increased after ischemia (AI) and decreased at the 15 min of reperfusion (AR) but these results are not statistically significant ( $p > 0.05$ ). Plasma NO levels of Group C were significantly increased when compared to baseline values and Group M at AI period. It decreased significantly in both of the groups AR. Plasma SOD levels in preischemia period increased significantly in both of the groups. Also decreased significantly compared to AI period, and increased significantly compared to basal values in both of the groups.

**Conclusions:** In conclusion 3 mg oral melatonin does not reduce I/R injury in skeletal muscle after pneumatic tourniquet application in human. It may reduce I/R injury in skeletal muscle in higher doses. So future investigation may be necessary to clarify if melatonin decreases I/R injury model of human skeletal muscle and at what dose or not.

**Keywords:** Biochemistry; Enzymes; Surgery; Orthopedics; Ischemia reperfusion injury; Melatonin

## Introduction

Pneumatic tourniquet application during vascular, muscular and arthroscopic surgical interventions results in prolonged extremity ischemia. Limb ischemia followed by reperfusion is an important clinical event and reperfusion of ischemic tissue with oxygenated blood plays an important role in the development of ischemia/reperfusion (I/R) injury [1]. As larger the mass of the ischemic tissue, such as legs, reperfusion results not only in local damage but also remote organ injury [2]. Melatonin (N-acetyl-5 methoxytryptamine) is a well-known antioxidant molecule and protector against ischemia reperfusion injury [3]. It is secreted from pineal gland in all mammals. It regulates circadian rhythms and sleep [4]. It decreases preoperative anxiety and causes sedation [5]. It has anticonvulsant and antiinflammatory effects [6].

Also oral melatonin decreases pain scores, enhances sleep quality, sedation scores, and subjective analgesic efficacy during the postoperative period [7]. It ameliorates hypoxia and reoxygenation induced damage [8]. It has a tissue protective effect against I/R injury [9].

In this randomized, prospective and double-blind study, it was aimed to evaluate the effect of oral melatonin, that is used for premedication, on ischaemia-reperfusion injury in patients undergoing arthroscopic knee surgery under spinal anaesthesia. Malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), haemodynamics, sedation scores were all measured for this purpose.

## Methods

After obtaining ethics committee approval and informed patient

consent, we studied 50 ASA physical status I or II patients, aged between 18 and 65, undergoing arthroscopic knee surgery requiring a pneumatic tourniquet. Patients were allocated randomly into two groups: control (group C, n=25), melatonin (group M, n=25), using the sealed envelope method. Patients with BMI of more than 30 kg m<sup>-2</sup>, severe hepatic or renal insufficiency, metabolic acidosis, congestive heart failure, intraoperative bleeding, hipo and/or hiperthermia, cigarette smoking and alcohol abuse, and those using antioxidant agents, pregnant, women in lactation period and people who were allergic to study drug were excluded from the study. No premedication other than melatonin or placebo was applied to the patients before surgery. Heart rate (HR), blood pressure (BP) and peripheral oxygen saturation (SpO<sub>2</sub>) were monitored in the operating room. Their sedation status was revealed with Ramsay Sedation Scale (RSS) [10]. The RSS defines the conscious state from a level 1: the patient is anxious, agitated or restless, through the continuum of sedation to a level 6: the patient is completely unresponsive.

An intravenous line inserted with 22 gauge cannula; 10 ml kg<sup>-1</sup> h<sup>-1</sup>

**\*Corresponding author:** Saritas TB, Assistant Professor, Department of Meram Medical School Anesthesiology and Reanimation, Necmettin Erbakan University, Konya, Turkey, Tel: +905443664244; Fax: +903322236181; E-mail: [tsaritas@konya.edu.tr](mailto:tsaritas@konya.edu.tr)

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0,09% NaCl started for the first hour and 5 ml kg<sup>-1</sup> h<sup>-1</sup> for following hours. Arterial catheter was inserted in the contralateral arm from the extremity with an intravenous (i.v.) line for blood sampling. Spinal anaesthesia was administered with 10-12.5 mg of 0.5% hyperbaric bupivacaine after a baseline (t1) blood sample had been obtained. After spinal anaesthesia was determined to be adequate by the use of a pinprick test at level of T8-10, surgeon is allowed to inflate the tourniquet. It is planned to apply 5 mg efedrin when patients' blood pressures decreased from 30% of basal values and 5 mg atropin heart rate lower than 45 min<sup>-1</sup>. Patients received either a placebo tablet (control group) or a 3-mg oral melatonin tablet (melatonin group: Melatonina tablet; (Przedsiębiorstwo Farmaceutyczne, Ostrzykowitzna, Zaczozym, Poland) 30 minutes before surgery. Tablets were given to the patients in an isolated quiet room in the operating suite by a nurse who was not involved in the study. The staff involved in data collection and patient management was unaware of the group assignments. Blood samples were obtained by another blinded observer immediately before spinal anaesthesia (t1), 30 min after tourniquet inflation (t2) and 15 min after tourniquet release (t3) for the measurement of MDA, SOD, NO. The samples were immediately centrifuged at 2500 g for 10 min and stored at -80°C until analysis All patients received 2 l min<sup>-1</sup> of oxygen through a nasal cannula. The tourniquet was applied at approximately twice fold of the systolic arterial pressure. Fluid deficits were corrected with 0.9% NaCl during surgery; no blood transfusion is required. Patients were followed postoperatively about side effects such as respiratory depression, drowsiness, nausea, vomit, headache, shaking and abdominal cramps.

### MDA analysis

Determination of MDA Level Plasma and tissue MDA levels were determined by a method based on the reaction with thiobarbituric acid (TBA) at 90-100°C. In the TBA test reaction, malondialdehyde (MDA) or MDA-like substances and TBA react with the production of a pink pigment having an absorption maximum at 532 nm. Results were calculated with using TBA-MDA extinction coefficient and the results were expressed in nanomoles per millilitre (nmol/mL) for plasma [11].

### NO analysis

The half-life of NO is only a few seconds. It is readily oxidized to nitrite (NO<sup>2-</sup>), and subsequently to nitrate (NO<sup>3-</sup>), which is used as index parameters of NO production [12,13]. The method for the measurements of nitrite and nitrate levels was based on the Griess reaction. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite plus nitrate) was measured by spectrophotometer at 545 nm after conversion of nitrate to nitrite by copperized cadmium granules. Results were expressed in micromoles per liter plasma (mmol L<sup>-1</sup>).

### SOD analysis

Determination of antioxidant enzymes SOD activity was measured kinetically by a method described by Sun et al. [14] and modified by Durak et al. [15].

The principle of the method is based on the inhibition of nitroblue tetrazolium reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was expressed as units per millilitre (Uml<sup>-1</sup>).

### Results

There were no significant differences between groups in terms of

age, weight, height, sex, ASA classification, BMI, tourniquet time and operation time, atropine and ephedrine need (Table 1). None of the patients need atropin or ephedrine during the operation. In the Control Group, 2 of the patients complaint about nausea at the postoperative period and with iv metoclopramid they were treated. Ramsay score 2 was statistically higher in Group M (p<0.05) (Table 2). Patients' mean heart rates were not statistically different between groups (p>0.05). Mean Respiratory rates was only higher at the basal measurement (p<0.05). Mean arterial pressures of the melatonin group were statistically lower after spinal block, 5, 10., and 20 minutes (p<0.05) (Figure 1).

Patients' baseline values of MDA, SOD and NO were not statistically different between the groups (p>0.05). Plasma concentrations of MDA increased at the post ischemia (PI) period and decreased at the 15 min of reperfusion (R) but these results are not statistically significant (p>0.05) (Figure 2).

Plasma NO levels of Group C were significantly increased when compared to baseline values and Group M at PI period (p<0.05). It decreased significantly in both of the groups R period (p<0.05) (Figure 3). Plasma SOD levels in preischemia period increased significantly in both of the groups (p<0.05) (Figure 4). Also decreased significantly compared to PI period, and increased significantly compared to basal values in both of the groups (p<0.05).

### Discussion

There are a lot of studies present in the literature on I/R injury and melatonin effect on skeletal muscle animal model. But the effects of oral melatonin on I/R injury on human skeletal muscle have not been evaluated yet. So we examined the effect of peroral 3 mg melatonin effect on MDA, NO, SOD levels in this human model of ischemia-reperfusion injury. We hypothesized that melatonin can decrease tourniquet induced acute muscular ischemia reperfusion injury.

The pneumatic tourniquet is frequently used for upper and lower extremity surgery to reduce bleeding, improve visualisation surgical site, decrease transfusion need and operation time and expedite surgical procedures. Although it is frequently used, it is not an innocent device. It has some local side effects such as localised tissue damage secondary to cuff compression, ischaemia-reperfusion injuries, neuropraxia, muscle strength loss, vascular dystrophy, tissue necrosis, compartment syndrome, hematoma and also systemic cardiac, renal and pulmonary complications [16-19].

The combination of these problems may affect outcome and contribute to prolonged hospitalization and higher costs and also unfortunately may cause death.

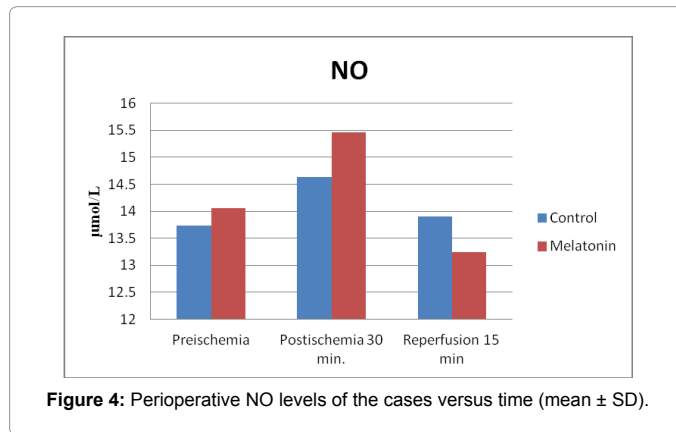
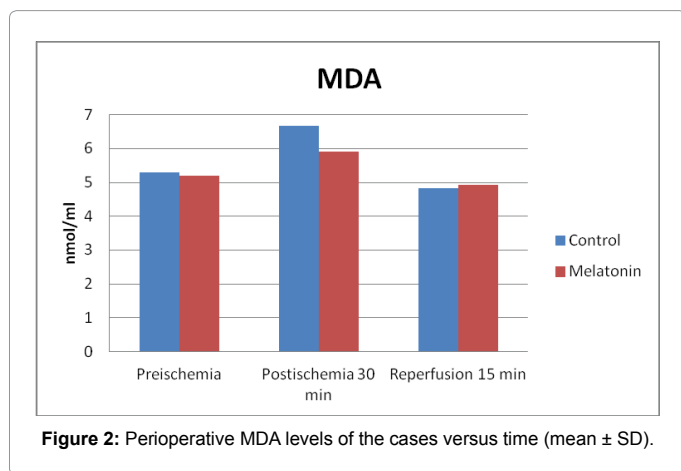
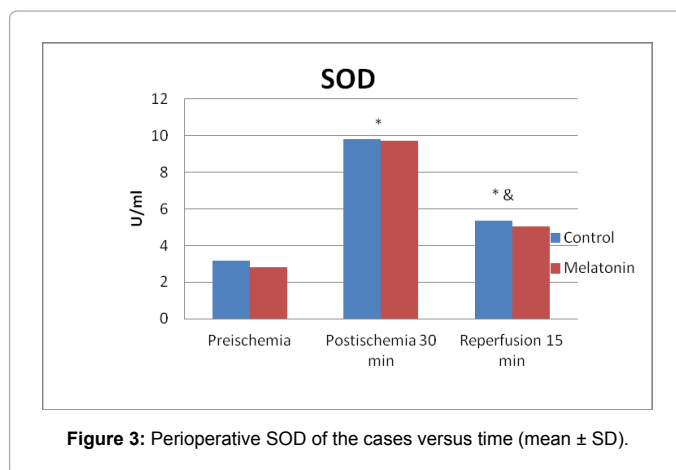
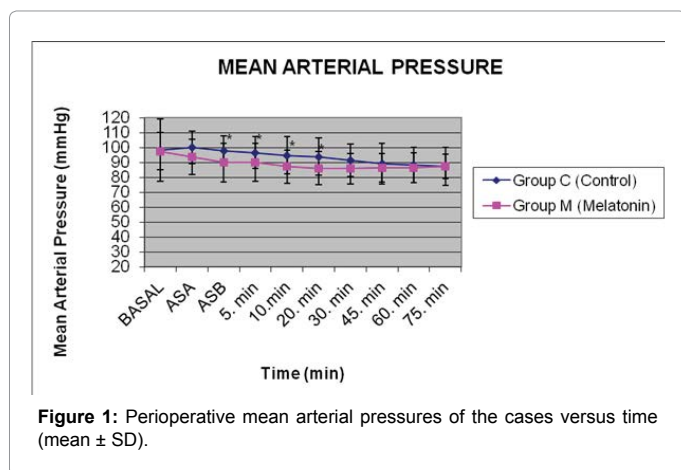
Aerobic organisms generate oxygen derived free radicals by using molecular oxygen. Free radicals have one or more unpaired electrons in their orbital and this increases their reactivity [20]. Superoxide anion radical, hydroxyl radical and nitric oxide are best known highly

	Group C (Control)	Group M (Melatonin)
Age (years)	46.24 ± 16.32	47.56 ± 14.25
BMI (kg m <sup>-2</sup> )	26.08 ± 2.09	26.16 ± 2.09
Sex (F/M)	11/14	12/13
Operation time (min)	64.80 ± 4.20	65.20 ± 4.44
Tourniquet time (min)	67.20 ± 5.01	67.00 ± 4.56
ASA(1/2)(n)	14/11	14/11
Ephedrine need	0	0
Atropine need	0	0

Table 1: Patient characteristics data are mean ± SD or number of the patients.

Time	Group C (n=25) 1/2/3/4/5/6	Grup M (n=25) 1/2/3/4/5/6	P value
BASAL	18/7/0/0/0/0/	19/6/0/0/0/0*	0,001
RAMSAY 5. Min.	16/9/0/0/0/0	3/22/0/0/0/0*	0,001
RAMSAY 10. Min.	15/10/0/0/0/0	3/22/0/0/0/0*	0,001
RAMSAY 20. Min.	10/15/0/0/0/0	2/23/0/0/0/0*	0,01
RAMSAY 30. Min.	8/17/0/0/0/0	1/24/0/0/0/0*	0,001
RAMSAY 40. Min.	9/16/0/0/0/0	1/24/0/0/0/0*	0,001
RAMSAY 60. Min.	12/13/0/0/0/0	0/25/0/0/0/0*	0,001
RAMSAY 80. Min.	12/13/0/0/0/0	0/25/0/0/0/0*	0,001

Table 2: Ramsay sedation scores of the cases (\*p<0.05 between groups).



reactive radicals and they can cause cellular dysfunction, toxicity and sometimes cell death [21]. These are called as reactive oxygen species (ROS). Oxygen based reactants and nitrogen derived species have been shown to play role in I/R injury on skeletal muscle [22,23]. Lipid peroxidation is one of the most important damaging effects of free radicals [24]. Malondialdehyde (MDA) is one of the toxic metabolites of lipid peroxidation after ROS production and can be used for free radical formation marker and reflects lipid peroxidation level [25,26].

Superoxide dismutase (SOD) reduced  $O_2$  to  $H_2O_2$ . It is an antioxidant enzyme that protects from free radical damage and it can be associated with increased oxidative stress [27]. In a lot of studies, SOD reported to be protective in I/R injury [28,29]. So higher level of MDA or lower level of SOD or both are indicative of peroxidative stress. Liu and Ng found that 5 mg/kg melatonin injection causes SOD enhancement in

rat liver, kidney and brain [30].

Ozturk et al. reported 10 mg/kg of melatonin administration for 7 days caused increased SOD activity in rat liver [31]. Saricaoglu et al. found that low-dose N-acetyl cysteine infusion in arthroscopic knee surgery attenuates I/R induced lipid peroxidation as measured by plasma MDA [32]. Also, Saricaoglu et al. studied the effect of ketamine sedation on oxidative stress by determining blood and tissue MDA and hypoxanthine levels and reported that ketamine sedation attenuates lipid peroxidation markers in arthroscopic knee surgery with tourniquet application [33]. In our study although MDA and SOD increased compared to basal (before ischemia) levels only SOD increase is statistically significant.

In nitric oxide synthesis nitric oxide synthase (NOS) is the key enzyme. NO interacts with superoxide anion and products peroxynitrite anion (OONO-) and this can degrade into -OH. So NOS may be

considered as prooxidative enzyme [34]. NO is believed to play role in ischemia reperfusion injury. NO is increased in both of the groups after ischemia compared to basal values and this increment is statistically higher in group M than control group. This result is unexpected it does not correlate with literature.

Several anesthetic drugs and methods were evaluated if they have effect on I/R injury or not. Yağmurdur et al. [35] examined the effect of dexmedetomidine on I/R injury in tourniquet applied upper extremity surgery by determining blood MDA and HPX levels.

They reported that dexmedetomidine significantly decreased plasma HPX production during ischemia and plasma MDA production compared to those in the control group after reperfusion. Koruk et al. compared the effects of dexmedetomidine and ketamine on hemodynamic and respiratory variables and on total anti-oxidant status, total oxidant status and MDA as markers of ischemia-reperfusion injury in patients undergoing arthroscopy under spinal anesthesia. They reported that dexmedetomidine had similar effects with ketamine in hemodynamic and respiratory variables during surgery, had comparable effects on ischemia-reperfusion injury and can be a safe alternative to ketamine as an intraoperative sedative [36].

Erturk reported that propofol and NAC infusions appear to provide protection against I/R injury in arthroscopic knee surgery [37]. Resveratrol, a polyphenol found in grape and red wine, was shown to have free radical scavenging and antioxidant properties in various tissues. Also shown to protect the skeletal muscles against I/R injury in rat hind limb [2].

The direct and indirect antioxidant effects of melatonin have been well established. Melatonin has been known as a potent free radical scavenger with the ability to remove reactive oxygen species (ROS) Melatonin also acts as an indirect antioxidant through the activation of major antioxidant enzymes including SOD, catalase, and glutathione peroxidase. Besides, free radical scavenging ability of melatonin. It has implications for a variety of diseases. It has been proposed that melatonin may have significant therapeutic effects. Melatonin has been used to attenuate jet lag and milder forms of insomnia [38]. Melatonin has been shown to have antiepileptic activity in animal studies and also in cases of childhood epilepsy [38].

Mantovani et al. reported that intraperitoneal, intraplantar, or intracerebroventricular route elicits significant and dose dependent antinociception in mice, when assessed in the behavioral model of nociception induced by an intraplantar injection of glutamate [39].

Melatonin is metabolized in the liver, converted to 6-hydroxymelatonin, and excreted in the urine as sulfate and glucuronide. Melatonin is quickly absorbed and also quickly excreted. Waldhauser et al. Administered 80 mg of melatonin administered orally to 5 young male volunteers, the peak serum melatonin levels were observed 60-150 min. after administration, and remained stable for about 90 min [40]. They also reported that the measured average biological half-life for the absorption from the gastrointestinal tract was 24 min., and a calculated average biological elimination half-life was 53 min. so we administered melatonin 30 min before the operation.

Wright et al. [41] have studied melatonin's bioavailability and pharmacokinetics in man and observed no adverse side effects for dose of daily 2 mg for one month (spring) and 3 weeks (autumn). So we used 3 mg oral dose in our study and we did not see any side effects in Melatonin group.

Although it has been proposed to have significant therapeutic

effects, the bioavailability of melatonin in humans is lower than rodents. This may be the one of the causes of our study results in which 3 mg oral melatonin is not effective in preventing I/R injury. In a study that using deuterium melatonin as a reference the bioavailability of orally consumed melatonin was as low as 1% in some subjects with an obvious sexual difference [42].

The bioavailability of melatonin in males is half that of females [43]. First pass effect through the liver may be the cause of low bioavailability. Although, the 3 mg oral dose of melatonin inhibits MDA elevation, stimulates SOD and inhibits NO, it is not enough to reduce I/R injury in skeletal muscle. This may be because of low oral dose or first pass effect.

In conclusion 3 mg oral melatonin does not reduce I/R injury in skeletal muscle after pneumatic tourniquet application in human. It is a widely used with variety of indications in humans. It may reduce I/R injury in skeletal muscle in higher doses. So future investigation may be necessary to clarify if melatonin decreases I/R injury model of human skeletal muscle and at what dose or not.

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