Original Article

Effect of Preoperative Administration of Oral Melatonin on Pneumatic Tourniquet-Induced Ischemia-Reperfusion Injury in Orthopedic Surgery of Lower Extremities: A Randomized Clinical Trial

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What's Known

- Pneumatic tourniquet-induced ischemia-reperfusion injury (IRI) causes cell damage and cellular dysfunction. Various measures have been proposed to reduce these adverse effects.
- Melatonin has anti-inflammatory and antioxidant properties.

What's New

- Melatonin has no significant effect on pneumatic tourniquet-induced IRI in orthopedic surgery of the lower limbs. However, it can provide effective sedation in patients undergoing such surgeries.

Abstract

Background: Ischemic reperfusion injury (IRI) causes cellular damage and dysfunction. The present study aimed to evaluate the effect of melatonin on pneumatic tourniquet-induced IRI in orthopedic surgery of the lower extremities.

Methods: A randomized clinical trial was conducted at Chamran Hospital, Shiraz University of Medical Sciences (Shiraz, Iran), from September to November 2019. The target population was patients scheduled for elective orthopedic surgery of the lower extremities. A total of 67 patients were randomly divided into two groups, placebo (n=34) and melatonin (n=33). The groups received 10 mg melatonin or placebo the night before surgery and two hours before surgery. Primary outcome variables were the serum levels of superoxide dismutase (SOD) and malondialdehyde (MDA). Hemodynamic parameters, sedation score, and drug side effects were also evaluated. Data were analyzed using SPSS version 21.0 software. $P<0.05$ was considered statistically significant.

Results: In the analysis phase, due to loss to follow-up (n=26), 41 patients divided into two groups of melatonin (n=20) and placebo (n=21) were evaluated. There was no significant difference in demographic data, duration of surgery (P=0.929), and tourniquet time (P=0.496) between the groups. The serum levels of SOD and MDA were not significantly different between the groups (P=0.866 and P=0.422, respectively), nor were the incidence of postoperative nausea (P=0.588) and patients' satisfaction (P=0.088). However, the postoperative sedation score and vomiting between the groups were significantly different ($P<0.001$).

Conclusion: Administration of 10 mg melatonin provided effective sedation, but had no significant effect on the serum levels of SOD and MDA, nor on pneumatic tourniquet-induced IRI in orthopedic surgery of the lower limbs.

Trial registration number: IRCT20141009019470N87

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Keywords ● Melatonin ● Superoxide dismutase ● Malondialdehyde ● Reperfusion injury

Introduction

Pneumatic tourniquets are typically used in surgeries involving the limbs, muscles, and arteries. The use of tourniquets has the advantage of reduced bleeding during surgery, improving the exposure of the operative field, and shortened operating time.[1] Despite these proven benefits, its use remains controversial due to potential complications such as neurological injuries ranging from transient neurological dysfunction to nerve paralysis.[2, 3] Common side effects include compartment syndrome, pressure ulcer, chemical burns, finger necrosis, deep vein thrombosis, tourniquet pain, and rhabdomyolysis.[3, 4] The use of tourniquets may also cause various adverse physiological effects, including progressive cellular hypoxia, acidosis, and cooling in the respective limb.[2] Muscle injury and elevated lactate concentrations can occur progressively in the respective limb after the inflation of the tourniquet cuff. Also, the return of blood supply after deflation may result in ischemia-reperfusion injury (IRI).[2, 3] Various preoperative precautions and intra-operative control measures have been suggested to reduce these adverse effects and to protect the limb from potential damages. Nonetheless, IRI occurs, particularly in prolonged surgeries, which necessitates alternative protective measures.[5, 6] Episodes of ischemia-reperfusion result in the production of oxygen-free radicals which leads to cell damage, cellular dysfunction, and eventually cell death.[7]

Malondialdehyde (MDA) is one of the toxic end-product of lipid peroxidation that leads to cellular injury and cell death. Superoxide dismutase (SOD) is an antioxidant enzyme present in all living cells exposed to oxygen, which converts superoxide-free radicals to oxygen molecules or hydrogen peroxide and protects tissues from damages associated with oxygen free radicals. It seems that the enzyme activity of SOD increases with the use of certain drugs such as melatonin.[8-10] Therefore, one of the current strategies for the prevention of IRI is an antioxidant therapy to reduce the level of oxygen free radicals in cells affected by cellular hypoxia.[11]

Melatonin (N-acetyl-5-methoxytryptamine) is an intracellular direct free radical scavenger. Its direct potent antioxidant and anti-inflammatory capacity is well known and has an indirect effect on antioxidant enzymes such as SOD and catalase. Melatonin is used in certain doses to reduce oxidative stress, especially in experimental studies on the spinal cord, heart, intestine, and skeletal muscle tissues.[12-17] Melatonin is naturally secreted by the pineal gland and plays a key role in regulating circadian rhythms, sleep-wake cycle, lifespan, and mood disorder.[18, 19] Some studies have examined the antioxidant effect of melatonin in addition to its role in psychiatric disorders and improving sleep quality.[20-22] Saritas and colleagues investigated the effect of oral melatonin in orthopedic arthroscopic surgery. They reported that 3 mg oral melatonin (a low dose) did not reduce pneumatic tourniquet-induced IRI in skeletal muscles and recommended further investigations with higher doses.[5] Hence, in the present study, a high-dose of oral melatonin (10 mg) was administered preoperatively to evaluate its effect on pneumatic tourniquet-induced IRI in orthopedic surgery of the lower extremities.

Materials and Methods

A randomized clinical trial was conducted at Chamran Hospital, Shiraz University of Medical Sciences (Shiraz, Iran), from September to November 2019. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1397.429) and registered in the Iranian Registry of Clinical Trials (IRCT 20141009019470N87). Written informed consent was obtained from each eligible participant.

In accordance with previous studies on MDA, t*he sample size was calculated* using mean (μ_1=10 and μ_2=8) and standard deviation (δ_1=0.3 and δ_2=0.2) after the deflation of tourniquets.[21] Accordingly, a sample size of at least 32 patients (16 in each group) was determined (power of 80% at 5% significance level).

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (\delta_1^2 + \delta_2^2)}{(\mu_1 - \mu_2)^2}$$

The inclusion criteria were patients aged 20 to 65 years, American Society of Anesthesiologists (ASA) physical status classification of I or II,[23] and those scheduled for elective orthopedic surgery in the morning using pneumatic tourniquets with a tourniquet time of 80 to 100 minutes. The exclusion criteria were patients with body mass index (BMI) >30 kg/m^2, any hepatic, cardiac, or renal dysfunction (Cr>1.5), a history of metabolic acidosis, risk of high bleeding during surgery requiring blood transfusion, smoking habit or alcohol addiction, and those currently pregnant or breastfeeding, using analgesics or antioxidant medication, with psychiatric disorders on medication, and anemia (hemoglobin of <10 g/dl).

Initially, a total of 96 patients were assessed for

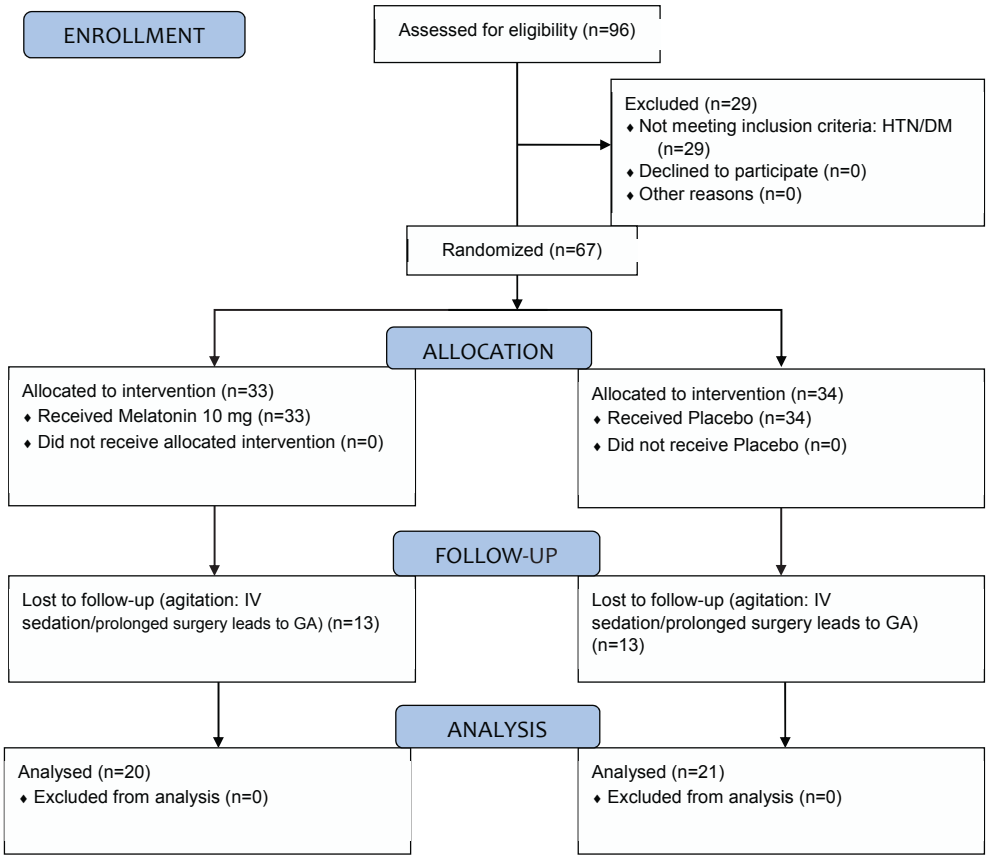

Figure 1: The CONSORT diagram shows the allocation process throughout the trial.

Table 1: Demographic characteristics and baseline data for the melatonin and placebo groups

Variable		Melatonin group (n=20)	Placebo group (n=21)	P value
Age (years)*		41.50±13.03	43.19±11.31	0.595
Sex (n, %)	Male	13 (65)	14 (66.7)	0.910
	Female	7 (35)	7 (33)	
BMI (kg/m^2)*		24.56±2.02	24.18±1.59	0.505
Duration of surgery (min)*		94.90±4.99	94.76±4.87	0.929
Tourniquet time (min)*		85.25±4.43	84.29±4.55	0.496
ASA physical status (n, %)	I	18 (90)	17 (81)	0.663
	II	2 (10)	4 (19)	
Usage of atropine (n, %)		5 (25)	7 (33.3)	0.558

*Data expressed as mean±SD; ASA: American society of anesthesiologists; BMI: Body mass index

eligibility, out of which 29 did not fulfill the inclusion criteria. The remaining 67 patients were randomly allocated into two groups, placebo (n=34) and melatonin (n=33), using a randomization list with a block size of four (sealedenvelope.com). In this process, confounding factors such as liver disease were omitted. In the analysis phase, 26 patients were lost to follow-up and the remaining 41 patients were included (figure 1).

The melatonin group received 10 mg melatonin (two 5 mg tablets) (Nature Made®, USA) and the placebo group received two inert starch tablets of the same shape and color. These were administered the night before surgery and two hours before surgery in the anesthetic induction room by a nurse anesthetist. The patients and all those involved (data collectors, laboratory personnel, statisticians, and nurses) were blinded to both study groups and medications. Demographic characteristics and baseline data included age, sex, duration of surgery, ASA physical status, tourniquet time, and usage of atropine (table 1).

Blood samples were collected by a nurse just before the intervention (T1: baseline), on the day of surgery immediately before spinal anesthesia (T2), 30 minutes after tourniquet inflation (T3), and 15 minutes after tourniquet deflation (T4). The SOD and MDA levels were then measured for both the placebo and melatonin groups.

The patients were placed in the supine position and standard monitoring was applied, namely electrocardiography (ECG), non-invasive blood pressure (NIBP), oxygen saturation (SpO_2), and capnography. All patients received 5-7 cc of normal saline prior to spinal anesthesia. Before applying the tourniquet, spinal anesthesia was performed in the sitting position by administrating 10-12.5 mg hyperbaric bupivacaine (0.5%). The adequacy of sensory block was determined at sensory points T8-T10 by using a pinprick test. In case of hypotension (more than 20-30% of baseline blood pressure), bradycardia, or heart rate less than 50 beats per minute, we administered 5 mg of ephedrine or 0.75 mg of atropine.

Primary outcome variables were the serum levels of SOD and MDA. Secondary outcomes were hemodynamic parameters, nausea, vomiting, and sedation scores based on the Ramsay sedation scale (RSS)[24] at baseline (prior to melatonin or placebo administration) and at time points of 5, 10, 20, 30, 45, 60, 80, 100 minutes after the medications were taken. The RSS divides a patient's level of sedation into six categories, namely (i) anxious and agitated, (ii) cooperative and tranquil, (iii) drowsy but responsive to commands, (iv) asleep but responsive to glabellar tap, (v) asleep with sluggish response to tactile stimulation, and (vi) asleep and not responsive. The RSS questionnaire was completed by a nurse anesthetist blinded to the study groups. Postoperatively, patients were evaluated for the side effects of melatonin such as respiratory depression, drowsiness, headache, and abdominal cramps.[25]

Statistical Analysis

Data were analyzed using SPSS software version 21.0 (SPSS Inc., Chicago, IL). Independent sample *t* test and Mann-Whitney U test were used to examine the difference between continuous variables and the results were expressed as mean±SD or median (interquartile range). The Chi square test or Fisher exact test was used to determine the difference between categorical variables and the results were expressed as number and percentage. Repeated measures ANOVA test was used to assess changes in outcome variables over time. P<0.05 was considered statistically significant.

Results

The results showed no significant differences in demographic characteristics and baseline data between the melatonin and placebo groups (table 1).

As shown in table 2, there was no statistically significant difference in nausea and patients' satisfaction between the groups (P=0.588 and P=0.088, respectively). However, the incidence of vomiting was significantly different between the groups (P<0.001). Patients in the placebo group had less vomiting compared to the melatonin group.

The results of the repeated measures ANOVA and univariate analysis showed no significant changes in MDA and SOD levels between the groups over time (P=0.422 and P=0.866, respectively) (figure 2). However, there was a significant change in the mean arterial pressure (MAP) (P<0.001) and sedation score (P<0.001) over time between the groups. Meaning that patients in the melatonin group had less MAP and higher sedation scores over time compared to the placebo group. However, duration had no effect on MDA (P=0.213) and SOD (P=0.100) levels.

Discussion

The effect of melatonin on pneumatic tourniquet-induced IRI was analyzed in 41 patients, which

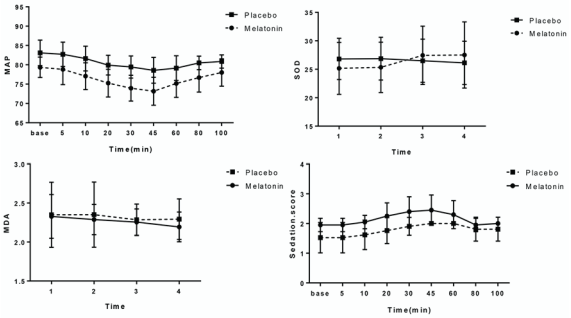

Figure 2: Changes in the mean arterial pressure (MAP), malondialdehyde (MDA), superoxide dismutase (SOD), and the score of Ramsey sedation scale (RSS) over time between the melatonin and placebo groups.

Table 2: Comparison of clinical variables between the melatonin and placebo groups

Clinical variable		Melatonin group (n=20)	Placebo group (n=21)	P value
Nausea (median, IQR)		0 (0-0.75)	0 (0-1)	0.588*
Vomiting (median, IQR)		0.5 (0-3)	0 (0-0)	<0.001*
Patients' satisfaction (n, %)	Satisfied	13 (65)	10 (47.7)	0.088#
	Neutral	6 (30)	4 (19)	
	Unsatisfied	1 (5)	7 (33.3)	

*Mann-Whitney U test; #Chi square test; IQR: Interquartile range

were randomly assigned to melatonin and placebo groups. Demographic characteristics (age, sex) and baseline data (BMI, duration of surgery, tourniquet time) were similar between the groups. The results showed that, except for vomiting, the difference in other clinical variables (nausea and patients' satisfaction) was not statistically significant between the groups. Moreover, there was no significant change in MDA and SOD levels over time between the groups.

Several studies have investigated the effect of melatonin on IRI. However, the majority have been animal experimentation and the effect on humans is scarcely evaluated.[5, 13, 16, 26, 27] Pneumatic tourniquets are commonly used in orthopedic surgery of the upper and lower limbs. One of its main side effects is IRI, which could affect surgery outcomes, hospital cost, and overall patient safety. Various drugs (melatonin, vitamin E, dexmedetomidine) and anesthesia plans have been proposed to reduce these adverse effects.[6, 28-30] In the present study, melatonin as an antioxidant agent was used to examine its effect on improving IRI.

The effect of melatonin on serum levels of MDA and SOD was examined at all predetermined time points and no significant changes were observed. Moreover, the changes between both groups were not statistically significant. In a previous study, Saritas and colleagues administered 3 mg oral melatonin to patients undergoing arthroscopic knee surgery using pneumatic tourniquets. They evaluated the antioxidant effect of melatonin by measuring the serum levels of SOD, MDA, and nitric oxide (NO). It was reported that such a low dose of oral melatonin (3 mg) did not reduce pneumatic tourniquet-induced IRI in skeletal muscles and recommended further investigations with higher doses.[5] In contrast, another study reported the positive effect of melatonin on MAD levels and IRI.[10] Other studies have reported the protective effect of melatonin and increased enzyme activity of SOD in the host tissue following the administration of melatonin.[8, 9] Possible reasons for the reported ineffectiveness of melatonin in reducing IRI could be due to the lower bioavailability of melatonin in humans than animals and its first-pass metabolism in the liver.[31-33] Other possible reasons for the poor results on melatonin could be due to the small study population, inappropriate dosage, or timing of melatonin administration.

Several studies have reported the sedative, anti-anxiety, and anti-inflammatory effects of melatonin in addition to its antioxidant effect on the heart, kidney, and intestinal tissues. Besides, melatonin can boost the immune system and plays a prominent role in regulating fluid nitrogen and acid-base balance.[11,13, 24, 34-39] In the present study, we found that the sedative effect of melatonin was statistically significant and a significant difference was observed between the melatonin and placebo groups. This was in line with the findings of previous studies that melatonin can reduce pain and anxiety levels and increase sedation.[24, 31]

The results of our study showed a statistically significant difference in MAP during surgery at a specific time point between the groups. However, patients in the melatonin group had a lower MAP compared to the placebo group. This could be due to the sedative, analgesic, and anti-anxiety effects of melatonin as well as attenuation of the sympathetic nervous system in humans after melatonin ingestion.[20, 24, 31] We monitored our patients for potential complications before and after the surgery. Overall, except for vomiting, the incidence of postoperative nausea and other complications were not significantly different between the groups.

The main limitation of the study was related to time restriction in enrolling the patients since those scheduled for elective lower limb surgery following spinal anesthesia could only be recruited the night before the surgery. Furthermore, despite strict exclusion criteria, other types of disease or medication could have influenced the level of oxygen free radicals.

Conclusion

Preoperative administration of 10 mg melatonin did not introduce significant changes in MDA and SOD levels over time. Therefore, it had no significant effect on pneumatic tourniquet-induced IRI in orthopedic surgery of the lower limbs. However, melatonin can provide effective sedation in patients undergoing such surgeries. Further studies with a larger sample size are required to comprehensively evaluate the antioxidant effect of melatonin in high doses.

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Conflict of Interest: None declared.

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