

Does the Circadian Rhythm of Melatonin Affect Ischemia-Reperfusion Injury after Coronary Artery Bypass Grafting?

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ABSTRACT

Background: Melatonin is a potent scavenger of free radicals and an antioxidant. We studied the relationship between the protective effect of melatonin against ischemia-reperfusion injury (IRI) during cardiopulmonary bypass, the plasma level of melatonin, and the time of surgery.

Methods: Forty patients who were to undergo elective coronary artery bypass grafting (CABG) were divided into 2 groups, those who underwent their operations at 8 AM (group I; n = 20) and those who underwent their operations at 1 PM (group II; n = 20). The operations were carried out by the same surgical team and with the same standard surgical technique. Blood samples were collected before the operation (T₁), when the aortic cross-clamp was removed (T₂), and at 4 hours (T₃) and 24 hours (T₄) after the operation.

Results: Preoperative plasma levels of melatonin were substantially higher in group I than in group II. Intraoperative and postoperative melatonin levels were also significantly higher in patients who underwent their operations in the morning. The 2 groups had similar preoperative levels of intercellular adhesion molecule 1 and interleukin 8; however, intraoperative and postoperative values were lower in group I for all samples. This difference was statistically significant for both markers. Plasma levels of lactate dehydrogenase were significantly lower in group I. The 2 groups had similar aortic cross-clamp and cardiopulmonary bypass times. Preoperative and postoperative troponin I levels were lower in group I than in group II, but these differences were not statistically significant. The 2 groups showed no significant differences in plasma creatine kinase MB levels for either preoperative or postoperative measurements.

Conclusion: High plasma levels of melatonin may be directly related to low levels of IRI markers. Melatonin may have a protective effect against IRI in CABG. This effect seems to be directly correlated with the plasma levels of

melatonin and inversely related with light. If melatonin protects myocardium from IRI, additional studies may be planned for the preoperative use of melatonin in patients with coronary artery disease to improve myocardial protection.

INTRODUCTION

Melatonin is a neurohormone that is secreted from the pineal gland with a circadian rhythm. Darkness stimulates the release of melatonin, and light suppresses its activity [Brzezinski 1997]. It adjusts the biological clock located at the suprachiasmatic nuclei of the anterior hypothalamus and plays an important role in human homeostasis [Nowak 1998]. Melatonin is also a known potent scavenger of free radicals and an antioxidant agent [Reiter 2003]. Clinical studies have shown that plasma melatonin levels do not differ between the sexes [Fourtillan 2001] and that melatonin secretion is reduced in coronary artery disease [Sakotnik 1999].

The purpose of this study was to show the protective effect of melatonin against myocardial ischemia-reperfusion injury (IRI) in coronary artery bypass grafting (CABG) surgery and its relation with the plasma level of melatonin and the time of surgery.

MATERIALS AND METHODS

Methods

After obtaining approval from the hospital research ethics committee and written informed consent, we included in the study 40 patients who were to undergo elective CABG. The patients were divided into 2 groups. The patients in group I (n = 20) underwent CABG in the morning, and the patients in group II (n = 20) underwent their operations in the afternoon. Demographic data for the patients are summarized in Table 1.

We excluded from the study elderly patients (>75 years), reoperations, patients with coexisting renal insufficiency, patients with serious pulmonary disease, those with prior stroke or significant cerebrovascular disease, and patients with an ejection fraction <40%. Patients undergoing CABG with concomitant heart valve repair or replacement, resection of a ventricular aneurysm, or other surgical procedures also were excluded. The 2 groups of patients were nearly homogeneous.

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Table 1. Preoperative Clinical Data of the Patients*

Variable	Group I (n = 20)	Group II (n = 20)	P
Male/female sex, n	18/2	14/6	NS
Age, y	58.1 ± 9.8	60.1 ± 9.2	NS
Hypertension, n	8	7	NS
Diabetes mellitus type I or II, n	4	4	NS
Acute myocardial infarction	0	0	NS
Ejection fraction, %	58.5 ± 7.2	56.5 ± 4.6	NS

*Data are presented as the mean ± SD, where indicated. NS indicates not statistically significant.

All of the operations in each group started at the same time, and all of the patients in the study underwent their operations with the same technique and by the same surgical team. The operations started regularly at 8 AM for morning cases and 1 PM for afternoon cases. All of the patients received the same cardiac drug regimen before the operation, generally angiotensin-converting enzyme inhibitors, β-blockers, vasodilators, lipid-lowering agents, aspirin, or heparin.

Group I consisted of 18 men (90%) and 2 women (10%), with a mean age of 58.1 ± 9.8 years (range, 42-75 years). Group II consisted of 14 men (70%) and 6 women (30%), with a mean age of 60.1 ± 9.2 years (range, 41-75 years). The 2 groups were not significantly different with respect to sex, age, additional diseases (hypertension, diabetes mellitus), or ejection fraction. None of the patients had acute myocardial infarction.

Surgical Technique

Patients underwent their operations on cardiopulmonary bypass with routine ascending-aorta and right-atrial cannulation. The core temperature was allowed to drift to 32°C,

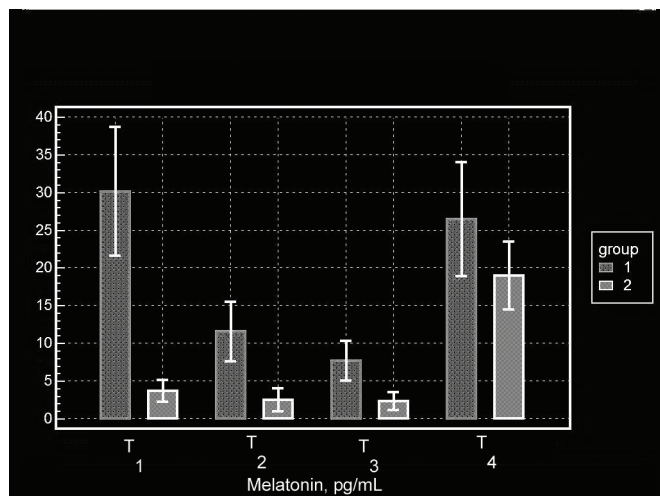


Figure 1. Melatonin values at consecutive time points. Data are expressed as the mean and the 95% confidence interval. Sampling times were preoperatively (T₁), when the aortic cross-clamp was removed (T₂), and at 4 hours (T₃) and 24 hours (T₄) postoperatively.

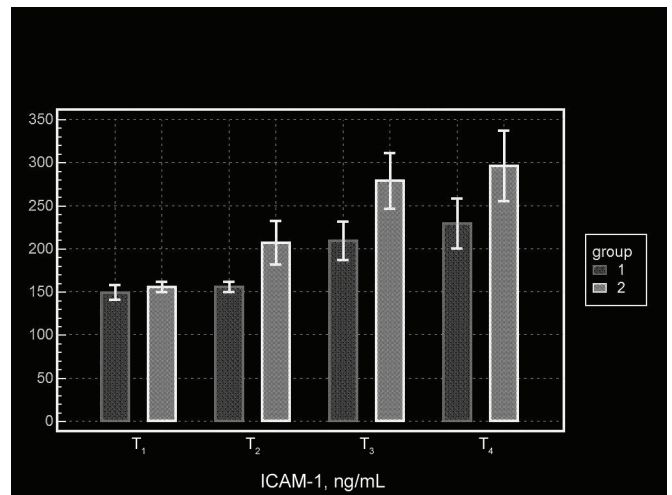


Figure 2. Intercellular adhesion molecule 1 (ICAM-1) values at consecutive time points. Data are expressed as the mean and the 95% confidence interval. Sampling times were preoperatively (T₁), when the aortic cross-clamp was removed (T₂), and at 4 hours (T₃) and 24 hours (T₄) postoperatively.

and aortic venting was performed. Myocardial protection was provided with antegrade and intermittent retrograde cold blood cardioplegia. Distal and proximal anastomoses were completed in a single aortic cross-clamp period.

Blood Sampling and Analysis

Blood samples were collected from the central venous line before the operation (T₁), when the aortic cross-clamp was removed (T₂), and at 4 hours (T₃) and 24 hours (T₄) after the operation. The plasma was separated by centrifugation at 1500g for 15 minutes at -4°C and stored at -70°C for later analysis. Concentrations of melatonin, intercellular adhesion molecule 1 (ICAM-1), interleukin 8 (IL-8), creatine kinase isoenzyme MB (CK-MB), lactate dehydrogenase (LDH), and troponin I (Tn I) were measured and recorded.

Melatonin levels in serum samples were measured with an enzyme immunoassay kit (DRG Instruments, Marburg, Germany). IL-8 and ICAM-1 levels were measured with human IL-8 and sICAM-1 ELISA kits (BioSource International, Camarillo, CA, USA). Plasma levels of Tn I were measured immediately with a Tn I immunoassay kit (Beckman Coulter, Fullerton, CA, USA). CK-MB measurements were made with an Olympus AU640 analyzer system (Olympus Life and Medical Science, Dublin, Ireland).

Statistical Analysis

Statistical analysis was performed with SPSS statistical software (version 11.0; SPSS, Chicago, IL, USA). Continuous variables were expressed as the mean ± SD, and differences were evaluated statistically with the Student *t* test for independent samples. Categorical variables were expressed as frequencies and analyzed with either the Pearson χ^2 test or the Fisher exact test, as appropriate. Differences between groups in consecutive measures were analyzed by repeated-measures analysis of variance. The Bonferroni correction was used for

Table 2. Significant Perioperative and Postoperative Data of the Patients*

Variable/Sampling Time	Group I	Group II	P†	P‡
Melatonin, pg/mL				
T ₁	30.1 ± 18.7	3.7 ± 3.1	.00004§	
T ₂	11.6 ± 8.7	2.5 ± 3.3	.0002§	.0002
T ₃	7.7 ± 5.7	2.3 ± 2.5	.001§	
T ₄	26.5 ± 16.5	19.0 ± 9.8	.091	
ICAM-1, ng/mL				
T ₁	149.4 ± 18.9	155.9 ± 13.13	.22	
T ₂	156.3 ± 13.3	207.5 ± 55.4	.0002§	.007
T ₃	209.4 ± 48.8	279.1 ± 71.4	.001§	
T ₄	229.6 ± 64.2	296.6 ± 90.1	.01	
IL-8, pg/mL				
T ₁	10.9 ± 1.95	11.7 ± 2.5	.27	
T ₂	16.5 ± 2.59	19.47 ± 2.9	.002	.03
T ₃	14.7 ± 2.38	18.3 ± 3.2	.0003§	
T ₄	14.2 ± 2.06	15.7 ± 1.7	.0018	
LDH, U/L				
T ₁	118.1 ± 103.6	190.5 ± 110.8	.039	
T ₂	166.8 ± 132.5	264.8 ± 130.7	.024	.27
T ₃	154.6 ± 119.9	271.0 ± 139.1	.007	
T ₄	170.6 ± 145.4	260.4 ± 136.0	.051	

*Data are presented as the mean ± SD. Sampling times were preoperatively (T₁), when the aortic cross-clamp was removed (T₂), and at 4 hours (T₃) and 24 hours (T₄) postoperatively. ICAM-1, intercellular adhesion molecule 1; IL-8, interleukin 8; LDH, lactate dehydrogenase.

†P values for the difference between groups I and II at the same time point.

‡P values determined by repeated-measures analysis of variance for differences at consecutive time points between groups.

§Indicates significant difference at time points with Bonferroni post hoc analysis.

post hoc comparisons. A *P* value <.05 was considered statistically significant (*P* < .0125, for a Bonferroni post hoc test).

RESULTS

Tables 2 and 3 summarize the perioperative and postoperative results for the patients.

Preoperative plasma levels of melatonin (T₁) were substantially higher in group I than in group II. Intraoperative and postoperative melatonin levels (T₂, T₃, and T₄) were also significantly higher in patients who underwent their operations in the morning (Table 2, Figure 1). Plasma melatonin levels were associated with the hormone's antioxidant effect.

The 2 groups had similar preoperative ICAM-1 levels, but intraoperative and postoperative values in group I were significantly lower in all samples (Table 2, Figure 2). The 2 groups

had similar preoperative IL-8 levels; group I had significantly lower IL-8 values at the other 3 measurement times (Table 2, Figure 3). Plasma LDH levels were significantly lower in group I than in group II (Table 2). Aortic cross-clamp and cardiopulmonary bypass times for the 2 groups were similar (Table 3).

Preoperative and postoperative Tn I levels were lower in group I than in group II, but the differences were not statistically significant (Table 3). The differences between the 2 groups in plasma CK-MB levels were not significant, for either preoperative or postoperative measurements (Table 3).

DISCUSSION

Despite improvements in anesthesia management, surgical technique, and postoperative care, CABG with cardiopulmonary bypass is associated with oxidative stress [Gerritsen 2001], and IRI is well known to be due to the accumulation of excess free oxygen radicals in the blood.

Melatonin was discovered to be a free radical scavenger more than a decade ago [Tan 1993]. The efficacy of melatonin as an antioxidant agent relates to its direct actions in scavenging free radicals, its ability to enhance the activities of a variety of antioxidative enzymes, its stimulatory effects on the synthesis of glutathione (which is also a strong antioxidant), and its synergistic interactions with other antioxidants [Reiter 2007]. It is also apparent that when melatonin scavenges free radicals, the generated products are also scavengers of free radicals. This process potentiates the antioxidant potential of melatonin [Hardeland 2005]. Melatonin's positive effect on IRI was evident in our analysis of plasma ICAM-1, IL-8, and LDH levels in the present study. Higher plasma concentrations of melatonin led to low plasma levels of IRI markers.

In this study, we chose ICAM-1 and IL-8 as markers of the inflammatory response. The plasma concentration of soluble

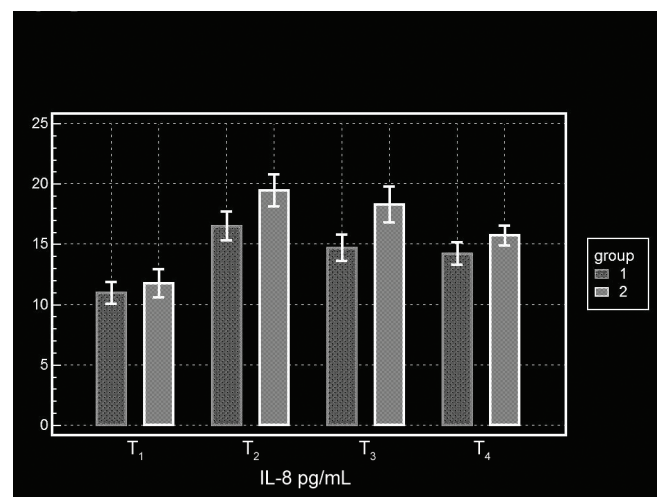


Figure 3. Interleukin 8 (IL-8) values at consecutive time points. Data are expressed as the mean and the 95% confidence interval. Sampling times were preoperatively (T₁), when the aortic cross-clamp was removed (T₂), and at 4 hours (T₃) and 24 hours (T₄) postoperatively.

Table 3. Nonsignificant Perioperative and Postoperative Data of the Patients*

Variable/Sampling Time	Group I	Group II	P†	P‡
Cross-clamp time, min	51.3 ± 25.5	43.2 ± 12.0	NS	
CPB time, min	68.4 ± 28.4	56.8 ± 15.4	NS	NA
Troponin I, ng/mL				
T ₁	0.01 ± 0.01	0.6 ± 2.5	NS	
T ₂	0.06 ± 0.06	1.2 ± 3.7	NS	.33
T ₃	0.04 ± 0.04	1.1 ± 3.3	NS	
T ₄	0.03 ± 0.04	0.59 ± 1.8	NS	
Creatine kinase MB, U/L				
T ₁	19.7 ± 9.9	17.0 ± 5.9	NS	
T ₂	37.1 ± 16.3	47.3 ± 20.6	NS	.06
T ₃	31.3 ± 18.9	32.2 ± 17.1	NS	
T ₄	27.2 ± 14.2	28.50 ± 13.1	NS	

*Data are presented as the mean ± SD. Sampling times were preoperatively (T₁), when the aortic cross-clamp was removed (T₂), and at 4 hours (T₃) and 24 hours (T₄) postoperatively. NS indicates not statistically significant; CPB, cardiopulmonary bypass; NA, not applicable.

†P values for the difference between groups I and II at the same time point.

‡P values determined by repeated-measures analysis of variance for differences at consecutive time points between groups.

ICAM-1 has been demonstrated to increase proportionally with the amount of myocardial destruction [Li 1997]. In addition, myocardial perfusion has been shown to initiate rapid induction of IL-8 in the previously ischemic tissue. Thus, IL-8 may mediate activation of neutrophil adhesiveness and motility in the context of myocardial perfusion [Kukielka 1995].

This study was performed with a small group of hemodynamically stable patients with good left ventricular function. Collection of blood samples was planned in detail, and emergency operations were excluded to obtain correct blood measurements from stable patients. Plasma Tn I and CK-MB levels could have been different if we had performed the study with hemodynamically unstable patients with poor left ventricular function.

Light is a strong timekeeper for the human circadian rhythm and influences several endocrine and neuroendocrine functions. On the other hand, melatonin is a neurohormone that reaches its peak level in darkness and becomes inactivated by light [Brzezinski 1997]. Logically, we expect higher plasma melatonin concentrations earlier in the day. In our study, melatonin concentrations showed an inverse correlation with light, and preoperative plasma melatonin levels of patients who underwent their operations in the morning were significantly higher than in the patients who underwent their operations in the afternoon.

Sakotnik et al [1999] showed that pineal production of

melatonin at night is significantly reduced in patients with angiographically documented coronary artery disease, compared with age-matched patients without coronary atherosclerotic changes. Furthermore, Guo et al [2002] demonstrated that melatonin levels were disturbed in patients who underwent CABG surgery with cardiopulmonary bypass. In our series, we also observed lower preoperative baseline melatonin levels. Postoperative plasma levels of melatonin were also significantly reduced in the 2 groups. When we compared the 2 groups, however, melatonin levels in group I were significantly higher than in group II. This finding indicates that melatonin secretion has a circadian rhythm even in patients with coronary artery disease, and plasma melatonin levels are higher at the beginning of the day.

Stoschitzky et al showed that β -blockers can suppress nocturnal melatonin secretion [Yin 2007]. On the other hand, it has been reported that patients with coronary artery disease who are not receiving β -blocker treatment already demonstrate a marked decrease in nocturnal melatonin synthesis and that introduction of a β -blocker does not induce significant further suppression of nocturnal melatonin secretion [Sakotnik 1999]. In our study, all patients received β -blocker treatment until the morning of the operation.

In conclusion, high plasma melatonin levels may be directly related to low levels of IRI markers. Melatonin may have a protective effect against IRI in CABG surgery. This effect seems to be directly correlated with plasma melatonin levels and inversely related with the strength of light. If melatonin protects the myocardium from IRI, additional studies may be planned for the preoperative use of melatonin in patients with coronary artery disease to improve myocardial protection.

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