The Effect of Circadian Melatonin Levels on Inflammation and Neurocognitive Functions Following Coronary Bypass Surgery

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Purpose: In this study, the relationship between the plasma levels of melatonin and intercellular adhesion molecule-1 (ICAM-1), which plays role in several intercellular interactions including inflammatory and immune responses, and early neurocognitive functions associated with ischaemia-reperfusion injury during open heart surgery is examined.

Methods: Forty patients who were to undergo elective coronary artery bypass grafting (CABG) were divided into two 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). Blood samples were collected prior to surgery (S1), when the aortic cross clamp was removed (S2) and 4 (S3) and 24 h after the surgery (S4). Neuropsychiatric assessment was conducted one day before and seven days after surgery.

Results: Melatonin levels measured during and after surgery were also significantly higher in Group 1. ICAM-1 levels were significantly lower in Group 1 at S2 and S3. Significant deterioration was observed in postoperative neurocognitive function compared with preoperative functions in Group 2 more than Group 1.

Conclusion: We hypothesise that the greater preservation of neurocognitive functions in the morning patients is associated with elevated melatonin levels, which reduce the damage from ischaemia-reperfusion injury.

Key words: coronary bypass, intercellular adhesion molecule-1, melatonin, neurocognitive functions

Introduction

Despite current advances in technology and surgical techniques, the rate of mortality from neurological complications associated with hypoperfusion and cerebral emboli following coronary artery bypass grafting (CABG) is between 7.2% and 19.6%.1,2) Although the mortality rate among patients developing neurological complications following CABG is 35.7%, this rate is only 4% in patients without neurological disorders.3) Cerebral injury following CABG may manifest itself as an overt neurological complication or as a postoperative cognitive dysfunction (POCD) that may not be discernible under
normal conditions. Several studies that comparatively evaluated POCD using different techniques CABG have demonstrated rates between 9% and 44% for impaired cognitive functions. This variation in reported rates most likely results from differences in the testing methodology and follow-up periods. Cerebral injury leads to a wide variety of impairments in emotional, cognitive, perceptual and behavioural functioning. Thus, a broad range of tests are available for different purposes. General skills, memory, perception and perceptual-motor performance, problem solving, language functions, orientation, attention and concentration of attention, behavioural flexibility and reaction time may all be assessed using different tests. Previous studies have reported that POCD associated with CABG is multifactorial including factors such as ischemic hypoperfusion brain lesions, microemboli, inflammatory responses, low body temperature during surgery.

Melatonin is a hormone produced by the pineal gland in all mammals and modulates the biological rhythm of living organisms. In addition to its established anti-inflammatory and antioxidant properties, melatonin produces a wide range of effects in an organism due to its lipophilic features; it crosses the blood-brain barrier and is able to access all organelles, including the cell nucleus. These properties render melatonin superior in protecting macromolecules, particularly mitochondria and DNA, from oxidative damage which leads to disorders associated with degenerative or oxidative alterations. As a member of the immunoglobulin superfamily of soluble adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) plays an important role in a variety of interactions between cells including inflammatory and immune responses. In the current study, we investigated the effect of melatonin on inflammation and currently controversial protective effect on cognitive impairment due to cerebral injury that may arise following CABG and inflammation.

Materials and Methods

Patients
This prospective, descriptive study was approved by the institutional ethics committee. Informed consent was obtained from all patients. Forty patients who were candidates for primary coronary artery bypass surgery were enrolled between January and August 2014 and were allocated into two groups based on the scheduled time of surgery: the first group was scheduled in the morning (08:00) and the second group was scheduled in the afternoon (13:00) (Group 1, n = 20; Group 2, n = 20). Patients over 75 years of age and patients with a history of a cerebrovascular or neurological disease, concomitant renal failure or endocrine disorders (including diabetes), a Mini-mental test (MMT) score of 24 or less, those currently receiving antidepressant or anxiolytic medication and patients with an acute lesion as demonstrated by cranial diffusion magnetic resonance imaging (MRI) following the operation were excluded. Patients in both groups were operated on by an identical surgical team using an identical surgical technique. Surgeries in the morning began exactly at 08:00, and those in the afternoon began at 13:00. Neurocognitive tests were conducted in the morning between 10:00 and 12:00 one day prior to the operation and again on postoperative day 7 for patients in both groups. People administering the test are made aware of schedule of the surgery.

Surgical technique
Patients underwent surgical operation using cardiopulmonary bypass with routine ascending-aorta and right-atrial cannulation. The core temperature was allowed to decrease to 32°C, 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 one hour before the operation (S1), when the aortic clamp was removed (S2), and 4 h (S3) and 24 h (S4) after the operation. The plasma was separated by centrifugation at 2000g for 15 min at −4°C and stored at −80°C for subsequent analysis. Melatonin levels in serum samples were measured using an enzyme immunoassay kit (DRG Instruments, Marburg, Germany). ICAM-1 levels were measured using human ELISA kits (Bio Source International, Camarillo, California, USA).

Cognitive tests
Neuropsychological tests included Turkish adaptations of tests recommended by the “Statement of Consensus on Assessment of Neurobehavioral Outcomes after Cardiac Surgery” and additional tests that were straightforward to administer. Tests were administered in the following sequence.

Mini-Mental State Examination (MMSE): This is a widely used, brief test, which is used to quantitatively evaluate cognitive functions during a standard neuropsychiatric
examination. This test consists of 11 questions with a maximum score of 30 points. The MMSE includes orientation, working memory, arithmetic calculations, recall and language tests. Scores under 24 points indicate pathology.

**Rey Auditory Verbal Learning Test (RAVLT):** This test provides information on spontaneous memory, attention, perception, memory protection, retrieval and identification processes. A list of 15 different words is read aloud to the subject five consecutive times in an identical order, and in each of these attempts, the subject is asked to recall the words without giving consideration to the reading sequence. For each attempt, the words recalled by the subject are noted. After the fifth attempt, an interference list, which also comprises 15 words, is read to the subject, and the subject is asked to recall it once. Following a 30-min interval, the examiner asks the subject to remember the words from the initial list that was read five times. While each word from an identification list is read aloud, the subject is asked to indicate if it belongs to the initial list or interference list or if it is a new word. The number of words retrieved by the subject following the first repetition is recorded as the instant memory score (RAVLT 1). The maximum learning score is determined by the maximum number of words retrieved by the subject from the list of 15 words following the repetition (RAVLT 2). The long-term recall score is determined by the number of words retrieved by the subject from the initial list after the 30-min interval (RAVLT 3).²²

**Digit Span Test:** The digit span forward test (DSFT) requires the subject to repeat a series of digits that have been orally presented to them in order; the maximal span of numbers is included in the analysis. The digit span backward test (DSBT) requires the subject to repeat a series of digits in reverse order; the maximal span of numbers is analysed.²³

**Trail Making A–B (TM-A-B):** The Trail Making test evaluates visual search speed, scanning, speed of processing, mental flexibility and motor speed. This test is sensitive to frontal lobe functions including working memory, planning, attention and executive functioning. Trail Making tests are influenced by performance, IQ and age of the individual. On average, it takes approximately 36 s to complete Part A and approximately 81 s to complete Part B by subjects 60 years of age with a high school education.²⁴

**Clock Drawing Test (CDT):** This test uses a 4-point scoring scale: Drawing of a closed circle/square/rectangle (outer frame of the clock) = 1 point; numbers are in the correct place and position = 1 point; all 12 numbers are present (no missing numbers) = 1 point; and hour and minute hands are in the correct positions (e.g., 11:10) = 1 point. Thus, the maximum achievable score in this version of the CDT is 4 points, whereas the lowest score is 0 points.²⁵

**Beck Depression Inventory (BDI):** The BDI is a 21-item self-reporting scale developed to measure the severity of depression. The Turkish version is a reliable and valid tool that is extensively used.²⁶ Each item is scored from 0–3. The total score varies between 0 and 63. BDI scores between 0–9 are considered as no depression, 10–18 as mild depression, 19–29 as moderate depression, and 30–63 as severe depression.

**Beck Anxiety Inventory (BAI):** The BAI is a 21-item, self-rated inventory used to determine the frequency of anxiety symptoms. In the BAI, the categories are normal (0–7), mild (8–15), moderate (16–25), and severe (26–63).²⁷

### Statistical analysis

Univariate analyses were performed to compare the baseline characteristics. Mann-Whitney U tests (for numerical non-normal variables) were used to compare the two surgical groups, and Wilcoxon tests (for numerical non-normal variables) were used to compare two repeated measurements. Chi-square tests (for categorical variables) and Spearman’s rank correlation coefficient (for numerical variables) were used to demonstrate relationships between variables. Mean and standard deviations and percentages were used for descriptive statistics. All analyses were performed in SPSS for Windows, version 22.0. A two-sided p value less than 0.05 was considered significant.

### Results

#### Demographic characteristic

A review of the preoperative demographic and perioperative characteristics of the patients did not show a significant difference between the two groups (Table 1).

#### Plasma melatonin and ICAM-1 levels

When plasma melatonin levels measured before, during and 4 and 24 h after surgery were compared between the groups, patients who underwent surgery in the morning (Group 1) were found to have significantly higher melatonin levels before, during and 4 h after the surgery compared with those of patients who underwent surgery in the afternoon (Group 2) (Table 2). This finding is consistent with the anticipated circadian rhythm of melatonin.
ICAM-1 plays an established role in the development of inflammation and atherosclerosis, and in this study, blood samples obtained from Group 2 patients during surgery and 4 h after surgery exhibited significantly higher ICAM-1 levels compared with those of Group 1 (Table 2).

**Evaluation of neuropsychiatric tests:**

Although Groups 1 and 2 did not differ in cognitive tests and anxiety and depression tests conducted preoperatively, Group 1 (the group including patients who underwent surgery in the morning who had higher melatonin levels) demonstrated significantly better results on the MMSE, RAVLT 1, RAVLT 2, RAVLT 3, DSBT, DSFT, TM-A and TM-B tests repeated on postoperative day 7 compared with Group 2; however, no significant between-group differences were observed in the CDT and anxiety and depression tests (Table 3).

When we compared the results of neuropsychiatric tests within each group preoperatively versus postoperatively, Group 1 demonstrated significantly worse scores on the postoperative MMSE, RAVLT 3, DSFT, TM-A and TM-B cognitive tests; however, their anxiety state was significantly better following surgery. Group 2 demonstrated worse scores on all postoperative tests except for the DSBT and CDT cognitive evaluation tests, whereas their postoperative anxiety state was better compared to the preoperative period, as observed for Group 1 (Table 3).

A negative correlation was found between the RAVLT1 test and anxiety only in Group 2 when the effects of anxiety and depression on the cognitive tests were examined ($r = -0.376$, $p = 0.018$).
Table 3  Preoperative and postoperative evaluation and within-group comparison of neuropsychological tests

<table>
<thead>
<tr>
<th>NPT</th>
<th>Preoperative</th>
<th>Group 1</th>
<th>Postoperative</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>27.2 ± 1.4</td>
<td>27.5 ± 1.3</td>
<td>26.8 ± 1.2*</td>
<td>24.1 ± 1.4**††</td>
</tr>
<tr>
<td>RAVLT 1</td>
<td>7 ± 1.16</td>
<td>6.65 ± 1.34</td>
<td>7.05 ± 1.27</td>
<td>5.2 ± 1.19**††</td>
</tr>
<tr>
<td>RAVLT 2</td>
<td>12.5 ± 1.08</td>
<td>11.95 ± 0.88</td>
<td>12.55 ± 1.14</td>
<td>10.65 ± 0.98**††</td>
</tr>
<tr>
<td>RAVLT 3</td>
<td>9.4 ± 1.23</td>
<td>9.15 ± 0.81</td>
<td>9.85 ± 1.38*</td>
<td>8.4 ± 1.04**†</td>
</tr>
<tr>
<td>DSFT</td>
<td>5.73 ± 0.73</td>
<td>5.45 ± 0.68</td>
<td>5.42 ± 0.6</td>
<td>5.2 ± 0.41*</td>
</tr>
<tr>
<td>DSBT</td>
<td>4.15 ± 0.58</td>
<td>3.75 ± 0.55</td>
<td>4 ± 0.56</td>
<td>3.5 ± 0.68**†</td>
</tr>
<tr>
<td>TM-A</td>
<td>48.45 ± 5.3</td>
<td>48.25 ± 5.88</td>
<td>50.05 ± 6.06*†</td>
<td>56.7 ± 6.4**††</td>
</tr>
<tr>
<td>TM-B</td>
<td>103.5 ± 9.5</td>
<td>100.35 ± 8.76</td>
<td>109.6 ± 8.4**††</td>
<td>116 ± 9.29**††</td>
</tr>
<tr>
<td>CDT</td>
<td>3.7 ± 0.47</td>
<td>3.65 ± 0.48</td>
<td>3.75 ± 0.44</td>
<td>3.45 ± 0.51</td>
</tr>
<tr>
<td>BAI</td>
<td>10.3 ± 4.57</td>
<td>10.9 ± 4.5</td>
<td>9.35 ± 3.8*</td>
<td>9.75 ± 4.42**†</td>
</tr>
<tr>
<td>BDI</td>
<td>10.8 ± 5.36</td>
<td>13 ± 4.77</td>
<td>10.55 ± 5.08</td>
<td>12.4 ± 4.46</td>
</tr>
</tbody>
</table>

Significant at \*p < 0.05 **p < 0.001 (preoperative and postoperative evaluation of neuropsychological tests). Significant at \†p < 0.05 ††<0.001 (Within-group comparison of preoperative and postoperative). NPT: Neuropsychological tests

Discussion

Neurocognitive dysfunction associated with cardiopulmonary bypass represents a major concern for heart surgeons because it adversely affects the quality of life of patients. Considerable challenges exist in the evaluation of neurocognitive functions. Thus, in this study, we selected tests that have been adapted and validated for use in the Turkish population that addressed attention, concentration, memory, visual-spatial skills and psychomotor speed; these tests were also straightforward to complete to avoid distraction of the patients. Considering that the emotional changes associated with hospitalisation, preoperative stress and an unfamiliar environment may also impair cognitive functions, depression and anxiety scales were completed for all patients concurrently with cognitive tests, and their potential effects on cognitive tests were included in the correlation analysis. Melatonin secretion follows a circadian rhythm that varies in different species. The circadian rhythm of melatonin secretion is of endogenous origin, which reflects the fact that stimuli originate from the suprachiasmatic nuclei; the synthesis and production of melatonin are stimulated in the dark and suppressed by light.\(^{28}\) Melatonin is one of the essential elements of an organism’s antioxidant defence system. Acting as an antioxidant and free radical scavenger, melatonin effectively protects cellular compartments against oxidative damage.\(^{29,30}\) Additionally, given the antioxidant and anti-amyloid properties of melatonin, it demonstrates a protective effect against \(\beta\)-amyloid (A\(\beta\)), which is implicated in the pathogenesis of Alzheimer’s disease (AD).\(^{31}\) Melatonin is a calmodulin antagonist that prevents myosin phosphorylation, and thus vascular smooth muscle contraction, by binding to the calcium-calmodulin complex at the intracellular level.\(^{32}\) The two melatonin receptor subtypes that have been identified in mammals have been designated as MT1 (1a) and MT2 (1b).\(^{33}\) Decreased MT2 immunoreactivity and increased MT1 immunoreactivity have been reported in the hippocampus of AD patients.\(^{34}\)

Endothelial adhesion molecules, including ICAM-1, are primarily involved in inflammatory reactions. ICAM-1 is normally released at a low concentration; however, its release may be increased by several mediators, including interleukin-1 (IL-1) and tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)).\(^{35}\) During ischaemia and reperfusion, pro-inflammatory cytokines that stimulate increased ICAM-1 release were shown to be increased in hypoxic endothelial cells.\(^{36,37}\) Melatonin was shown to block ICAM-1 release in a variety of studies that investigated its vascular effects.\(^{37,38}\) Potential neurological and neuropsychological complications following cardiac surgery are categorised into two major groups. Type 1 complications (with an incidence of 3%–6%) include death due to stroke or hypoxic encephalopathy, non-fatal incident stroke, transient ischaemic attack, stupor and coma. Type 2 complications (40%–60%) include new-onset intellectual disability, confusion, agitation, disorientation, memory deficit and seizures (without focal injury).\(^{39}\) Although Type 1 complications primarily result from embolic events and hypoxia, Type 2 complications are associated with microembolization and systemic
inflammatory reactions.\(^{30}\) Cerebral hypoperfusion, which develops as a result of simultaneous haemodynamic impairment and associated systemic inflammation, represents a major causal factor in the development of neurological complications.\(^{41,42}\)

In this study, consistent with the circadian rhythm of melatonin, melatonin levels were higher in the group of patients who underwent surgery in the morning compared with the group who underwent surgery in the afternoon, as expected.

In this study, we chose to utilise ICAM-1 as the inflammatory marker. Plasma ICAM-1 levels have been shown to increase in direct proportion to cardiac damage.\(^{43}\) In our study, the two groups did not significantly differ in preoperative plasma ICAM-1 levels. However, significantly lower plasma ICAM-1 levels were observed during surgery and 4 h after surgery among patients undergoing surgery in the morning. At 24 h after surgery, ICAM-1 levels remained low in the morning group; however, a significant difference was not observed at this time point between the two groups. ICAM-1 is known to peak in the first hour of inflammation and this level increases during ischaemia and reperfusion and decreases at a later phase.\(^{44}\) Melatonin therapy has been previously shown to diminish ICAM-1 release.\(^{37}\) Consistent with this finding, reduced ICAM-1 levels were observed during surgery and 4 h after surgery in the group of patients with elevated melatonin levels who underwent surgery in the morning. At 24 h after surgery, no significant difference in the melatonin or ICAM-1 levels was observed between the two groups.

We hypothesise that melatonin may lower the incidence of cognitive dysfunction, which is an undesirable event that is frequently associated with coronary bypass surgery, via its antioxidant, anti-amyloid and vasodilator actions. Although no significant difference was observed between the two groups in preoperative neurocognitive tests and in the depression and anxiety scales, a significant decrease was observed in the scores of the majority of the neurocognitive tests (all tests excluding DSFT and CDT) when they were readministered at 7 days after surgery. However, the depression and anxiety scales did not exhibit differences at this timepoint. Less deterioration was observed in the cognitive tests of the group undergoing surgery in the morning when within-group comparisons were conducted individually preoperatively and postoperatively. Improvement in the postoperative anxiety scale was more significant compared to the preoperative period among patients who underwent surgery in the morning; however, a negative correlation with anxiety was observed in only one cognitive test (RAVLT1). Although previous animal studies have reported a positive effect of melatonin on cognitive functions,\(^{45-47}\) neurocognitive tests and melatonin levels following cardiopulmonary bypass surgery were evaluated in only one study, which reported findings similar to our findings;\(^{48}\) however, the present study was conducted in human subjects to explore the levels of melatonin and its effects on inflammation and neurocognitive functions.

However, there were certain limitations in the present study, including that the Patients’ demographics and perioperative data show no significant differences, but their p values are variable and we do not have long-term follow-up results of patients.

In conclusion, among patients undergoing cardiopulmonary bypass, decreases in neurocognitive scores were observed in both groups of patients regardless of the time of surgery (whether in the morning or in the afternoon). However, the morning group exhibited higher melatonin levels, a finding which was consistent with the circadian rhythm of melatonin, and less deterioration in cognitive functions, as measured by relevant tests, due to the antioxidant, anti-inflammatory, anti-amyloid and vasodilator effects of melatonin. No significant association was found with melatonin for the negative effects of anxiety and depression on cognitive functions.

**Disclosure Statement**

The Authors declare that there are no conflicts of interest.

**References**


