Effect of oral melatonin on the procoagulant response to acute psychosocial stress in healthy men: a randomized placebo-controlled study

Abstract: Acute mental stress is a potent trigger of acute coronary syndromes. Catecholamine-induced hypercoagulability with acute stress contributes to thrombus growth, after coronary plaque rupture. Melatonin may diminish catecholamine activity. We hypothesized that melatonin mitigates the acute procoagulant stress response and that this effect is accompanied by a decrease in the stress-induced catecholamine surge. Forty-five healthy young men received a single oral dose of either 3 mg melatonin (n = 24) or placebo medication (n = 21). One hour thereafter, they underwent a standardized short-term psychosocial stressor. Plasma levels of clotting factor VII activity (FVII:C), FVIII:C, fibrinogen, D-dimer, and catecholamines were measured at rest, immediately after stress, and 20 min and 60 min post-stress. The integrated change in D-dimer levels from rest to 60 min post-stress differed between medication groups controlling for demographic and metabolic factors (P = 0.047, \( \eta^2_p = 0.195 \)). Compared with the melatonin group, the placebo group showed a greater increase in absolute D-dimer levels from rest to immediately post-stress (P = 0.13; \( \eta^2_p = 0.060 \)) and significant recovery of D-dimer levels from immediately post-stress to 60 min thereafter (P = 0.007; \( \eta^2_p = 0.174 \)). Stress-induced changes in FVII:C, FVIII:C, fibrinogen, and catecholamines did not significantly differ between groups. Oral melatonin attenuated the stress-induced elevation in the sensitive coagulation activation marker D-dimer without affecting catecholamine activity. The finding provides preliminary support for a protective effect of melatonin in reducing the atherothrombotic risk with acute mental stress.

Introduction

Acute mental stress has been identified as an important trigger of acute coronary syndromes (ACS) [1–3]. After rupture of an atherosclerotic coronary plaque, the balance between prothrombotic and antithrombotic forces in the circulation determines the extent of intracoronary thrombus growth and consequent myocardial damage [4]. Catecholamine surge that accompanies triggering events may increase the risk of plaque thrombosis [2] as an acute systemic increase in catecholamines evokes a hypercoagulable state [5]. Particularly, epinephrine infusion to healthy individuals activates platelets and increases plasma levels of factor VIII coagulant activity (FVIII:C) and of hemostatically active von Willebrand factor [5]. Also, the magnitude of norepinephrine surge during acute mental stress was directly associated with increases in thrombin/antithrombin III complex (i.e. a marker of thrombin formation) [6] and D-dimer (i.e. a marker of fibrin formation) [7]. Acute mental stress was also shown to elicit an increase in plasma levels of factor VII coagulant activity (FVII:C) and fibrinogen [8]. Together with tissue factor exposed to the blood stream at sites of endothelial lesions, FVII initiates the extrinsic pathway of blood coagulation, ultimately leading to a fibrin clot further downstream in the coagulation cascade. During this process, thrombin activates FVIII and converts fibrinogen to fibrin [9].

Melatonin is a substance with pleiotropic physiologic actions synthesized in the pineal gland [10]. With regard to the emerging function of melatonin in cardiovascular disease [11], nocturnal secretion of melatonin was lower in patients with coronary artery disease, particularly in those with ACS, than in healthy controls [12–15]. Research suggests that exogenous administration of supraphysiologic melatonin dosages might favorably affect inflammatory and prothrombotic mechanisms pertinent to the manifestation of ACS. In animal models, melatonin reduced leukocyte rolling and adhesion in the microcirculation during acute inflammation [16] and normalized shortened prothrombin time and elevated levels of fibrin degradation products inflicted by thermal injury [17]. Melatonin infusion also reduced arrhythmias induced by experimental ischemia of the isolated rat heart [18]. In humans, platelet aggregation in vitro was inhibited by melatonin suggesting a
dose–response effect [19, 20]. Importantly, placebo-controlled studies found decreased plasma levels of norepinephrine 60 min after intake of 2 mg melatonin in healthy young men [21] and 5 min after intake of 1 mg melatonin in healthy young women [22] and men [23], respectively.

To our knowledge, it has not been investigated whether exogenous melatonin affects coagulation and catecholamine responses to acute mental stress. The above research suggests that oral melatonin could mitigate the procoagulant response to acute mental stress and that such an effect could be a consequence of reduced catecholamine activity during stress with melatonin medication. We specifically hypothesized that the integrated, stress-induced increase in plasma levels of FVII:C, FVIII:C, fibrinogen, and D-dimer from rest to 60 min post-stress is smaller with melatonin than with placebo. We controlled for age, body mass index (BMI), screening blood pressure (BP) and heart rate (HR) – as a proxy measure of vagal tone – as these variables may affect hemostatic activity in healthy subjects [24–27]. We further hypothesized that, in the melatonin group, attenuation of the procoagulant stress response is paralleled by a decrease in the catecholamine response to stress.

**Materials and methods**

**Participants and study design**

The study protocol was formally approved by the ethics committee of the State of Zurich (Switzerland) and by Switzerland’s regulatory agency for therapeutic products (Swissmedic, Bern, Switzerland). The study was carried out in accordance with the Declaration of Helsinki principles. All participants provided written informed consent. We report on 45 medication-free healthy young men, all nonsmokers, with a complete dataset in terms of coagulation and catecholamine measures. Subjects with any self-reported acute or chronic somatic or psychiatric disorder were not eligible for the study. Specific exclusion criteria were regular strenuous exercise, alcohol abuse and illicit drug use, heart disease, elevated blood sugar and diabetes, elevated cholesterol, liver and renal diseases, chronic obstructive pulmonary disease, allergies and atopic diathesis, rheumatic diseases, and current infectious diseases. We contacted the subjects’ primary care physician if the history was not conclusive.

All subjects were scheduled on 2 consecutive days. They abstained from food and drink other than water for 2 hr before reporting to the laboratory and from physical exercise, and consumption of alcohol and caffeinated beverages starting the evening before day 1. On day 1, after subjects had been sitting quietly for 20 min, screening BP and HR were measured once using the fully automated Omron device (Omron 773, Omron Medizintechnik Handelsgesellschaft mbH, Mannheim, Germany). The BMI was calculated by dividing the weight in kilograms by the square of height in meters (kg/m²). On day 2, testing sessions commenced between 14:00 and 16:00 hr. A venous catheter was placed and 25 min thereafter subjects were random-ized, single-blinded, to one oral pill of either 3 mg melatonin or placebo. Melatonin was purchased from Physiologics (“Melatonin Standard”, PhysioLogics, Northglenn, CO, USA). Placebo pills contained lactosis and amyllum (lactosi cum amylo) and were purchased from Hänseler AG (Herisau, Switzerland).

**Stress experiment**

Following previous observations on melatonin kinetics after oral intake of 1–5 mg of melatonin, subjects underwent the psychosocial stressor 60 min after intake of the medication, when blood levels of melatonin are expected to peak [28, 29]. We presumed that melatonin exerted physiological effects during the entire stress experiment as exogenously increased melatonin levels return to baseline values only within 4–8 hr [10].

We applied the standard protocol of the widely used Trier Social Stress Test (TSST), which combines a short introduction phase followed by a 5-min preparation phase, a 5-min mock job interview, and a 5-min mental arithmetic task in front of an audience [30]. The TSST evokes reliable increases in the coagulation measures investigated in the present study and in plasma catecholamines [7, 8]. During the 60-min recovery from the stressor, subjects remained seated in a quiet room. Blood samples for coagulation and catecholamine measures were collected at rest, immediately post-stress, and 20 min and 60 min thereafter. BP and HR were measured at rest and immediately post-stress using the Omron device. Data were missing for BP in five subjects and for HR in one subject.

**Biochemical analyses**

For coagulation measurements, 4.5 mL of blood was added to 0.5 mL of citrate, 0.106 mol/L. Samples were immediately centrifuged for 20 min at 3000 g at room temperature. Plasma aliquots were frozen at −80°C in polypropylene test tubes pending further analysis. FVII:C and FVIII:C were determined by standard coagulometric methods using factor-deficient standard human plasma and reagents (Dade Behring, Liederbach, Germany). Fibrinogen was quantified as per a modified Clauss method [31] and D-dimer levels by use of an enzyme-linked immunosorbent assay (Asserachrom Stago, Asnières, France). For the determination of plasma catecholamine and melatonin concentration, blood was drawn into EDTA-coated monovettes (Sarstedt, Numbrecht, Germany) and immediately centrifuged for 10 min at 2000 g at 4°C; plasma was stored at −80°C until analysis. Epinephrine and norepinephrine levels were determined by means of high-performance liquid chromatography and electrochemical detection after liquid–liquid extraction [32]. Melatonin was extracted with methylene chloride and analyzed with a reversed phase HPLC with fluorescence detection [33]. The lower limit of detection (LOD) for the melatonin concentration was 2.5 pg/mL. Samples with a melatonin concentration below the LOD were set at half the LOD (i.e. 1.25 pg/mL) following previous recommendation for a skewed dataset [34]. Inter- and intra-assay coefficients of variation were <10% for
all biochemical analyses (i.e. hemostatic factors, melatonin, catecholamines).

Statistical analysis

SPSS version 13.0 for Windows was used to analyse the data. Significance level was set at $P < 0.05$ (two tailed). Normal distribution of data was tested by the Kolmogorov–Smirnov test. In order to approximate a normal distribution, the values of FVIII:C, D-dimer, epinephrine, and melatonin were log transformed. Data are given as mean or geometric mean (for logged transformed variables) with S.D. in the text and with S.E.M. in the tables and figures. Student’s $t$-test was used to calculate differences in variables between two groups. Pearson’s correlation analysis was applied to estimate the bivariate correlation coefficient between two variables. Partial correlation analysis was used to control bivariate correlations for covariates.

As sphericity was violated in our dataset, we applied multivariate repeated measures analysis of covariance (MANCOVA) to test whether stress-induced changes in coagulation and catecholamine measures would differ between the melatonin and placebo group controlling for covariates. The four time points (i.e. rest, immediately post-stress, 20 min post-stress, and 60 min post-stress) were treated as the within-subjects variables and the medication group (i.e. melatonin or placebo) as the between-subjects factor. Roy’s largest root criterion was applied to test the significance of the multivariate test statistics. The number and type of covariates (i.e. age, BMI, BP, and HR) were selected a priori given our sample size [35] and based on the previous literature on variables possibly affecting hemostatic function [24–27]. In order to preserve degrees of freedom [35], the screening mean arterial pressure (MAP) was computed by the formula $2/3 \times$ systolic BP plus $1/3 \times$ systolic BP and included as a control variable in the analysis. We also controlled for resting levels of coagulation and catecholamines measures to account for potential differences between medication groups. Differences in resting levels of these parameters could unduly inflate a floor (i.e. resting level is comparably low) or ceiling (i.e. resting level is comparably high) effect in the stress response of coagulation and catecholamine measures. Effect sizes of the repeated measures MANCOVA are expressed as partial eta squared ($\eta^2_p$). Post hoc analysis applied Fisher’s least significant difference.

Results

Table 1 shows that age, BMI, BP, and HR were not significantly different between groups. Plasma levels of coagulation and catecholamine measures were determined 1 hr after intake of either placebo or melatonin corresponding to resting levels with regard to the stress experiment. At this time, plasma melatonin levels were expectedly higher in subjects who received melatonin than in those who received placebo medication ($140.9 \pm 386.0$ pg/mL vs. $1.7 \pm 1.6$ pg/mL, $P < 0.001$). Of note, the absolute plasma melatonin levels showed a wide range in subjects who had received melatonin (17.3–2046.4 pg/mL) as opposed to those who had received placebo (1.3–8.6 pg/mL).

Catecholamine levels were not significantly different between the two medication groups and showed no significant correlation with melatonin levels. As previously reported [36], resting FVIII:C levels were significantly lower in subjects who had received melatonin than in those who had received placebo (82.1 ± 32.8% vs. 101.1 ± 42.0%; $P = 0.048$). There was also a trend towards statistical significance for lower resting fibrinogen levels in the melatonin group compared with the placebo group ($1.98 \pm 0.29$ g/L vs. $2.20 \pm 0.50$ g/L; $P < 0.08$). Melatonin levels correlated with FVIII:C levels ($r = −0.31$, $P = 0.036$) but not significantly so with any other coagulation measure.

Table 2 presents the crude changes in hemodynamic indices, catecholamines, and coagulation measures from rest to immediately post-stress across all subjects. There was a significant increase in all hemodynamic measures and both catecholamines. Also, FVII:C, FVIII:C, and fibrinogen significantly increased in response to the TSST with a similar trend towards statistical significance observed for D-dimer. Altogether, these data verify that the TSST provoked a robust biologic stress response.

Fig. 1 shows that the integrated change in epinephrine and norepinephrine across all time points was not significantly different between groups controlling for catecholamine level at rest, age, BMI, MAP, and HR. This suggests that melatonin did not affect the response in catecholamine levels to stress and the recovery of catecholamine levels from stress differently from placebo medication.

Fig. 2A–C shows that the integrated change in FVII:C, FVIII:C, and fibrinogen levels between rest and 60 min post-stress was not significantly different between medication groups controlling for coagulation factor levels at rest, age, BMI, MAP, and HR. This suggests that melatonin affected neither the immediate response in these coagulation factors to stress nor the recovery of coagulation factor levels from stress differently from placebo medication.

In contrast, there was a significant interaction between time and medication for D-dimer levels controlling for

<table>
<thead>
<tr>
<th>Variable</th>
<th>Melatonin (n = 24)</th>
<th>Placebo (n = 21)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>24.3 ± 0.7</td>
<td>25.1 ± 0.8</td>
<td>0.446</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.0 ± 0.6</td>
<td>22.7 ± 0.4</td>
<td>0.683</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>94.1 ± 1.7</td>
<td>94.7 ± 2.2</td>
<td>0.828</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127.8 ± 3.0</td>
<td>124.8 ± 2.6</td>
<td>0.459</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.3 ± 1.6</td>
<td>79.7 ± 2.3</td>
<td>0.390</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>72.0 ± 2.3</td>
<td>65.6 ± 2.5</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E.M.; group comparison was by Student’s $t$-test.
resting D-dimer level, age, BMI, MAP; and HR ($F_{3,36} = 2.92, P = 0.047; \eta_p^2 = 0.195$). Fig. 2D illustrates that the integrated generation of D-dimer from immediately pre-stress to 60 min thereafter was significantly greater in the placebo group than in the melatonin group. Post hoc analysis controlled for resting D-dimer level, age, BMI, MAP, and HR; it revealed an absolutely greater increase in D-dimer levels from rest to immediately post-stress ($P = 0.13; \eta_p^2 = 0.060$) with a significant recovery from immediately post-stress to 60 min thereafter in the placebo group relative to the melatonin group ($P = 0.007; \eta_p^2 = 0.174$).

We recalculated the above repeated measures MANCOVA replacing medication group as the between-subjects factor by absolute levels of plasma melatonin as a covariate. Melatonin levels were not significantly associated with the integrated change in plasma levels of epinephrine ($P = 0.54$), norepinephrine ($P = 0.39$), FVII:C ($P = 0.87$), FVIII:C ($P = 0.61$), and fibrinogen ($P = 0.87$) controlling for the respective levels of coagulation measures and catecholamines at rest, age, BMI, MAP, and HR. There was a trend towards statistical significance for an association between melatonin level and D-dimer change from rest to immediately post-stress ($r = -0.29, P < 0.07$), from immediately post-stress to 60 min thereafter ($r = -0.35, P = 0.025$), and also from 20 min after stress to 60 min post-stress ($r = -0.32, P = 0.046$). No such significant associations were observed in either medication group alone.

**Discussion**

Consistent with previous studies in other samples, we found that the TSST provoked a significant stress response in coagulation measures and catecholamines [6–8], suggesting our protocol was suitable to test our hypotheses. In this randomized, placebo-controlled experimental study, we found some evidence for our primary hypothesis that melatonin, in an exogenously administered supraphysiological dose, attenuates the procoagulant response to acute psychosocial stress in humans. Independent of demographic and metabolic covariates, the integrated change in D-dimer from rest to 1 hr after stress was significantly lower in the melatonin group than in the placebo group. Although not reaching statistical significance, the absolute increase in D-dimer from rest to immediately post-stress was greater with placebo than with melatonin. This immediate D-dimer increase was mirrored by a significantly greater decrease in D-dimer levels in the placebo group.
relative to the melatonin group during the recovery phase from immediately post-stress to 1 hr thereafter. This observation was confirmed when testing for an association between absolute levels of plasma melatonin and stress-induced D-dimer change, i.e. the lower the plasma melatonin level 1 hr after intake of the medication, the greater the increase in D-dimer from rest to immediately post-stress and the greater the subsequent decrease in D-dimer during the recovery period from stress. Altogether this suggests that subjects who received placebo had significantly greater fibrin formation during acute stress and the subsequent recovery phase of 1 hr than subjects who received melatonin. Moreover, there might be an inverse dose–response relationship between plasma melatonin levels and D-dimer formation with stress. Noteworthy, the dispersion of plasma melatonin levels in subjects who received melatonin was high. This could perhaps be a consequence of differences in metabolic clearance between subjects. Future studies may want to investigate the extent to which individual clearance of melatonin might affect the procoagulant response to acute stress.

D-dimer is a marker of coagulation activation indicating both fibrin formation and its subsequent degradation by the fibrinolytic system [37]. In stress experiments, elevated D-dimer levels in plasma indicate that the entire coagulation system has been activated during stress [38]. As opposed to elevated activity levels of individual clotting factors, coagulation activation markers are more sensitive to a hypercoagulable state [39]. This could be an explanation for why we did not find a significant difference between the melatonin and placebo group in terms of their stress responses in FVII:C, FVIII:C, and fibrinogen. Thus, supraphysiological melatonin dosages might attenuate the procoagulant stress response in vivo at different and likely subtle levels throughout the entire coagulation and fibrinolysis pathways rather than overtly at the level of individual hemostatic factors. We acknowledge, however, that we did not measure platelet activity previously shown to be inhibited by melatonin in vitro [19, 20]. Therefore, we are unable to discount the possibility that the decreased D-dimer change to stress with melatonin was largely a consequence of platelet inhibition. Moreover, we did not

---

Fig. 2. The stress response in D-dimer (D) was significantly different between the melatonin group and the placebo group, whereas the stress response in factor VII:C (A), factor VIII:C (B), and in fibrinogen (C) did not significantly differ between groups. All analyses controlled for coagulation factor level at rest, age, body mass index, mean arterial blood pressure and heart rate. Values are given as mean ± S.E.M. for factor VII:C and fibrinogen and as geometric means for factor VIII:C and D-dimer.
measure body temperature, which has been shown to decrease in a dose-dependent manner within 30 to 60 min after administration of melatonin dosages between 1 mg and 5 mg [40]. Also, although not investigated in our study, acute mental stress reliably increases tissue-plasminogen activator (t-PA) activity, thereby enhancing fibrinolytic activity [41]. Fibrinolytic activity in vitro was found to be reduced when t-PA is relatively high and when body temperature is relatively low [42]. The attenuated D-dimer formation in our subjects who received melatonin could therefore reflect relatively attenuated fibrinolytic activity and consequently less fibrin degradation with acute stress because of melatonin-induced lowering of body temperature.

Rupture of a coronary plaque and subsequent thrombosis marks the core transition from stable coronary artery disease to ACS [4]. Previous studies reported higher D-dimer concentrations in ACS than for control subjects reflecting that D-dimer is a plasma marker of activated hemostasis in the early phase of ACS [43]. Increased D-dimer concentration during admission for ACS predicted major adverse cardiac events during hospitalization [44]. The coagulation activation by stress adds to the prothrombotic state initiated by plaque rupture thereby arguably increasing the overall risk of clinical manifestation of ACS [41, 45]. Hence, our data suggest that the blunting effect of melatonin on the procoagulant response to acute psychosocial stress is clinically meaningful. Indeed, medication explained almost 20% of the variance in stress-induced D-dimer change. An anticoagulant effect of melatonin would also concur with a previous study showing that melatonin infusion reduced ischemic complications after experimentally induced myocardial ischemia in the isolated rat heart [18].

In our subjects, melatonin did not affect the catecholamine response to acute psychosocial stress differently from placebo. Therefore, the present study does not support our secondary hypothesis that reduced D-dimer formation in the melatonin group was mediated by attenuated catecholamine activity. In contrast to other placebo-controlled studies on healthy young men [21, 23], we did not observe lower plasma norepinephrine levels in the melatonin group compared with the placebo group 1 hr after intake of the medication. This could be because we obtained blood for catecholamine measurements while subjects were sitting, whereas previous studies found an effect of melatonin on plasma norepinephrine levels only when subjects were supine [21–23]. Also, subjects were relaxed in previous studies, so anticipatory arousal before the experiment could have blurred a group difference in our subjects. However, we feel it is premature to definitely conclude that melatonin does not exert anticoagulant properties during stress by reducing catecholamine activity.

The randomized, placebo-controlled design and application of a standardized stress protocol provoking reliable biologic stress responses were strengths of our study. Moreover, our study design probably prevented biasing of D-dimer findings by diurnal variations in circulating D-dimer levels [46]. Our data cannot be generalized to women, elderly subjects and patients with coronary artery disease who are the at-risk population for ACS. However, it seemed prudent to first investigate the effect of melatonin on coagulation activation with stress in a healthy young population before subjecting patients with cardiovascular diseases to a study medication (i.e. melatonin) that has not been approved by Switzerland’s regulatory agency for therapeutic products. Because we were unable to confirm a catecholamine hypothesis explaining attenuated coagulation activity with stress after melatonin administration, the mechanisms by which melatonin may decrease the acute procoagulant stress response are subject to future studies. For instance, markers of a hypercoagulable state may also reflect the endogenous anticoagulant properties of the vascular endothelium [39]. Melatonin and several of its metabolites have antioxidative properties and the capacity to regulate vascular tone, both of which alone or in combination might relate to endothelial anticoagulant function [10, 47–53].

Taken together, we found that a single dose of oral melatonin attenuated the stress-induced change in plasma D-dimer levels relative to placebo. Although the stress reactivity of individual clotting factors was not affected by melatonin, our data lend some support to the hypothesis that melatonin might mitigate overall activation of the coagulation system (i.e. fibrin turnover) during stress. Because melatonin did not attenuate catecholamine reactivity, the physiological mechanisms involved in the anticoagulant effect of melatonin during stress are currently speculative. It also remains to be determined whether melatonin may alleviate the increased risk of ACS with acute mental stress.

Acknowledgments

This study was financially supported by research grant 2004 from the University of Zurich to P.H.W., and by a research grant from the University of Bern to R.v.K. The authors are grateful to André Haebeli and Monika Stutz for laboratory assistance.

References


363


34. **Hornung R, Reed L.** Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg* 1990; **5**:46–51.


42. **Yenari MA, Palmer JT, Bracci PM et al.** Thrombolysis with tissue plasminogen activator (tPA) is temperature dependent. *Thromb Res* 1995; **77**:475–481.


