EFFECTS OF MELATONIN AND LUZINDOLE ON PLASMA LEVELS OF TISSUE FACTOR, TISSUE FACTOR PATHWAY INHIBITOR AND VON WILLEBRAND FACTOR IN RATS

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ABSTRACT

INTRODUCTION: The role of melatonin in hemostasis is still poorly studied.

PURPOSE: The purpose of this study was to investigate the effects of melatonin and luzindole, inhibitor of the melatonin receptors – MT_1 and MT_2, on plasma levels of tissue factor, tissue factor pathway inhibitor and von Willebrand factor.

MATERIAL AND METHODS: The study was performed on 52 male Wistar rats, kept at 12/12 h natural regimen dark/light. The daily doses of melatonin 0.2 mg/kg b.m. and luzindole 0.4 mg/kg b.m. were applied subcutaneously twice daily at intervals of 12h for three consecutive days. The rats were distributed into four equal groups (n=13) and were treated as follows: the first group (control group) – by saline; the second group – by melatonin; the third group – by luzindole; and the fourth group – by luzindole and an hour later by melatonin.

RESULTS: The results show that melatonin significantly increases the plasma levels of TF, reduces the values of free TFPI antigen and free TFPI activity, and increases the values of vWF antigen and vWF activity. Applied alone, luzindole lowers the plasma levels of TF, increases the values of free TFPI antigen and free TFPI activity; decreases the values of vWF antigen and vWF activity. Pretreatment with luzindole repeats the effect of self-administration. The received data show that melatonin induces a pronounced tendency to hypercoagulability in rats by a significant increase in TF, a decrease of free TFPI and free TFPI activity, as well as an increase in vWF antigen and vWF activity.

CONCLUSIONS: Lizindole self-administration and pretreatment show a decisive involvement of MT_1 and MT_2 receptors for accomplishing the effects of the hormone.

Keywords: *melatonin, luzindole, TF, TFPI, vWF, MT_1 and MT_2 receptors, hypercoagulability*

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INTRODUCTION

Tissue factor (TF), also known as plasma factor III, is a transmembrane glycoprotein which plays an initiating role for blood coagulation in the extrinsic pathway (1,2). It is widely present in all tissues but is expressed mostly in subendothelial cells, adventitial fibroblasts, alveolar cells, cardiomyocytes, astrocytes, trophoblasts, and others (3,4,5). The tissue fac-

Scripta Scientifica Medica, vol. 47, No 1, 2015, 64-69

tor has a high affinity to the VIIa factor and together they form the TF/FVIIa complex (tenase complex), which activates IX and X factors, and thus, the coagulation cascade to form fibrin (6,7).

The tissue factor pathway inhibitor (TFPI) is a major physiological regulator of the TF-induced coagulation (8,9). Its biosynthesis is associated primarily with the microvascular endothelium (10,11). The TFPI molecule contains three consecutive Kunitz-type protease inhibitor domains, and the second domain (K2) connects and inhibits the Xa factor, and the first (K1) – with the tenase complex (TF/FVIIa) (12,13). As a result of this, a quaternary complex TFPI – TF/FVIIa – FXa is formed, which is inactive (14). This complex is the basis of the regulatory effect of TFPI on the initiated by the TF intrinsic pathway for thrombin generation (1,15).

The Von Willebrand factor (vWF) is a glycoprotein, which performs two key functions in normal hemostasis. The first – mediates the adhesion of platelets to the subendothelial matrix after vascular injury (16,17) and the second – protects the VIII factor from proteolytic degradation (18). vWF is released into circulation by secretion from endothelial cells (accumulates in Weibel-Palade bodies) and platelets (their alpha granules) (19,20). It is well known that abnormalities in platelet plug and fibrin formation are observed in patients with von Willebrand disease (17).

PURPOSE

The purpose of this study was to investigate the effect of melatonin and luzindole (melatonin receptors inhibitor – type 1 and 2) on plasma levels of tissue factor, tissue factor pathway inhibitor and von Willebrand factor.

MATERIAL AND METHODS

Experiments were carried out on 52 white male Wistar rats, weighing 200–220 g, kept at natural regimen dark/light 12/12 h in accordance with European Convention and Directives (Protection of animals used for experimental purposes, Council Directive 86/609/EEc of November 1986, Directive 2010/63/EU of the European Parliament and of the Council of September 2010). Rats were fed by standard food and water ad libitum. The daily doses of melatonin (Merck, Germany) 0.2 mg/kg b.m., and luzindole (Sigma Chemicals, USA) – 0.4 mg/kg b.m. were applied s.c. twice daily at intervals of 12h, for three consecutive days. The upper doses were determined experimentally in preliminary studies and according to literature data. The rats were distributed into four equal groups (n=13) and were treated as follows: the first group (C) (control group) – by saline; the second group – by melatonin (M); the third group – by luzindole (L); and the fourth group – by luzindole and an hour later by melatonin (L+M).

The necessary blood volume from a rat was received by cardiac puncture under urethane narcosis in single usage plastic tubes, using sodium citrate 0.11 mol/l (blood citrate ratio 9:1) as anticoagulant.

TF was determined by an American Diagnostica, inc. test (USA), and by performing Diagnostica Stago tests (France), the following were determined: free TFPI, free TFPI activity, vWF antigen (vWF:Ag) and vWF activity, defined by collagen binding capacity (vWF:CB capacity).

Rats were autopsied and examined for macroscopic haemorrhages. Preparations from internal organs were stained by hematoxylin-eosin and for fibrin by Weigert, and inspected for microhaemorrhages and intravascular coagulation.

STATISTICAL ANALYSIS

Data were processed by variation analysis using Student–Fisher's t-test on GrafpadPRYSM 4.2 software. Values of $p< 0.05$ were considered significant.

RESULTS

Results are presented in Fig. 1–5. Fig. 1 shows that melatonin increases plasma levels of TF (pg/ml) from 120.30±6.29 to 159.80±10.42, ($p<0.01$). Luzindole self-administration and luzindole pretreatment with a subsequent injection of melatonin, decreases the values of TF to 42.23±3.05 ($p<0.001$) and 52.31±6.340 ($p<0.001$), respectively. Changes in plasma levels of free TFPI (ng/ml) (Fig. 2), indicate that melatonin decreases its values from 11.87±0.54 in the control group – to 5.50±0.46 ($p<0.001$), luzindole increases them to 15.48±1.06 ($p<0.01$), while pretreatment with luzindole – to 16.03±0.85 ($p<0.001$).

Melatonin reduces the free TFPI activity (Fig. 3) from 93.41±6.96 in controls – to 53.19±9.29 ($p<0.01$), luzindole increases it to 146.50±11.05 ($p<0.001$), as does the pretreatment with luzindole – to 153.40±9.31 ($p<0.001$).

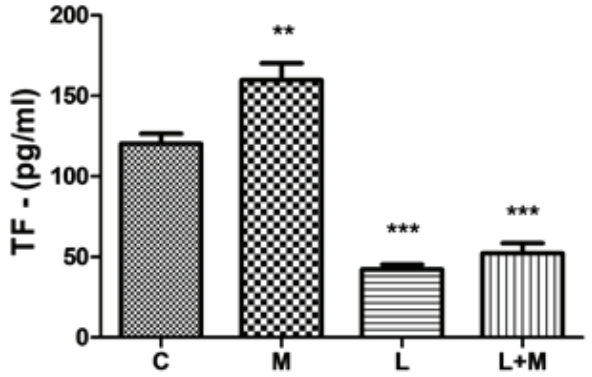

Fig. 1. Effects of melatonin (0.2 mg/kg b.w.) and luzindole (0.4 mg/kg b.w.), applied s.c. to male Wistar twice daily at intervals of 12h, for three consecutive days on TF level (pg/ml). TF – Tissue factor; C – control group, injected by saline; M – melatonin; L – luzindol; L+M – luzindol followed after 1 h by melatonin
*** $p < 0.001$; ** $p < 0.01$

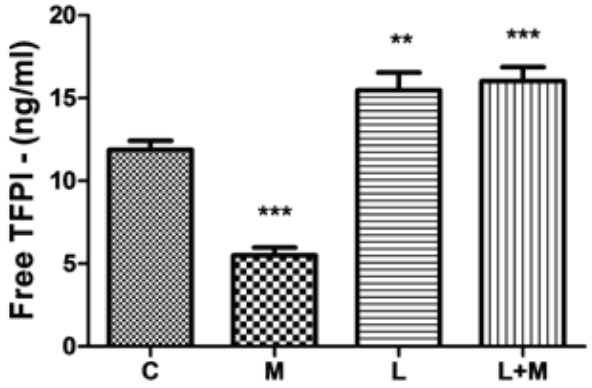

Fig. 2. Effects of melatonin (0.2 mg/kg b.w.) and luzindole (0.4 mg/kg b.w.), applied s.c. to male Wistar twice daily at intervals of 12h, for three consecutive days on free TFPI (ng/ml). Free TFPI – free Tissue factor pathway inhibitor; C – control group, injected by saline; M – melatonin; L – luzindol; L+M – luzindol followed after 1 h by melatonin
*** $p < 0.001$; ** $p < 0.01$

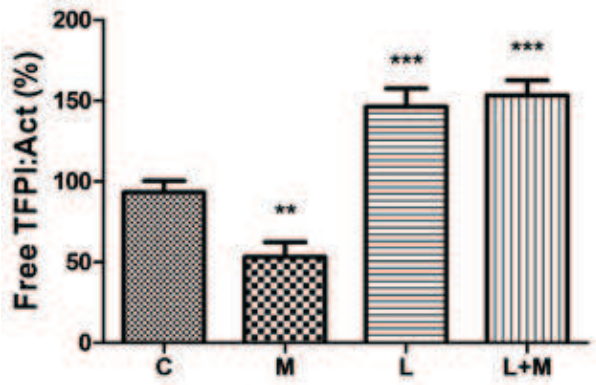

Fig. 3. Effects of melatonin (0.2 mg/kg b.w.) and luzindole (0.4 mg/kg b.w.), applied s.c. to male Wistar twice daily at intervals of 12h, for three consecutive days on activity free TFPI (%). Free TFPI:Act – free Tissue factor pathway inhibitor activity; C – control group, injected by saline; M – melatonin; L – luzindol; L+M – luzindol followed after 1 h by melatonin
*** $p < 0.001$; ** $p < 0.01$

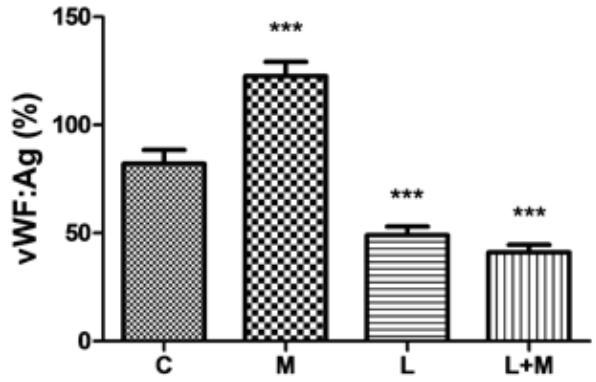

Fig. 4. Effects of melatonin (0.2 mg/kg b.w.) and luzindole (0.4 mg/kg b.w.), applied s.c. to male Wistar twice daily at intervals of 12h, for three consecutive days on VWF antigen (%). VWF:Ag – von Willebrand factor, antigen; C – control group, injected by saline; M – melatonin; L – luzindol; L+M – luzindol followed after 1 h by melatonin
*** $p < 0.001$

Plasma levels of vWF antigen (Fig. 4) under the influence of melatonin are highly elevated (122.50±6.63, p<0.001) compared to the control group rats (82.01±6.42). Treatment with luzindole was followed by a reduction of vWF antigen levels to 49.01±4.05 (p<0.001). Strong reduction was also observed after pretreatment with luzindole (41.10±3.41, p<0.001).

The vWF activity increases under the influence of melatonin to 167.70±9.65 (p<0.01) (Fig. 5), relative to controls (127.10±5.79). Luzindole reduces the activity to 68.91±5.15 (p<0.001), while pretreatment with luzindol – down to 60.54±3.04 (p<0.001).

DISCUSSION

Fig. 1 shows that melatonin, administered to the rats, causes a significant increase in plasma levels of TF (p<0.01) compared to the control group. Since TF is known primarily as a trigger of the extrinsic coagulation pathway and thrombogenesis in a

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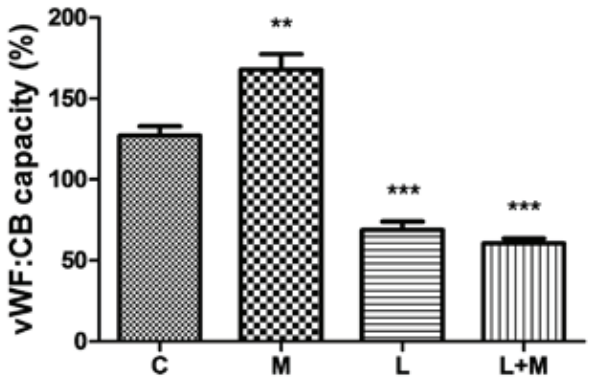

Fig. 5. Effects of melatonin (0.2 mg/kg b.w.) and luzindole (0.4 mg/kg b.w.), applied s.c. to male Wistar twice daily at intervals of 12h, for three consecutive days on activity vWF:CB (%). vWF:CB capacity – activity vWF as estimated by collagen binding capacity; C – control group, injected by saline; M – melatonin; L – luzindol; L+M – luzindol followed after 1 h by melatonin
**** p < 0.001; ** p < 0.01*

number of diseases (1,2,21), it could be assumed that melatonin induces a tendency to hypercoagulability. This assumption is supported by our previous study (22) where it was shown that this hormone violates the equilibrium between the coagulant and anticoagulant system in favor of the coagulant. The foregoing analysis allows to assume that increased plasma levels of TF under the influence of melatonin are a part of the mechanism explaining the tendency to hypercoagulability, which is convincingly shown in the already published article. Self-administration of luzindole strongly reduces the TF levels, ($p<0.001$), which gives grounds most likely to assume that blocking the melatonin receptors (MT_1 and MT_2) removes to a large extent the effect of endogenous melatonin on structures associated with the formation of this factor. We cannot exclude the fact, albeit hypothetically, that luzindole may inhibit somewhat the biosynthesis and secretion of endogenous melatonin, which is relevant to the final effect. For now, there are no published data on the level of melatonin in plasma after administration of luzindole. Of particular interest are the results from the fourth group of animals, where melatonin is administered after pretreatment with luzindole. On the one hand, the results repeate those from the third group (luzindole only). On the other hand, it shows that blocking the melatonin receptors suppresses the effect of endogenous and exogenous melatonin, i.e. these results are new evidence for the role of melatonin receptors as well as the importance of endogenous melatonin in TF formation.

The results presented in Fig. 2 and Fig. 3 are a logical continuation of those in Fig. 1, showing the changes in TFPI, also known as major physiological inhibitor of TF-mediated coagulation cascade (1,8,9). Based on this fact and the well-known mechanism of TFPI action (12-15) there is reason to assume that the significantly lower levels of free TFPI (Fig. 2) ($p<0.001$) and especially free TFPI activity (Fig. 3) ($p<0.01$) under the influence of melatonin, are inevitably accompanied by development of a tendency to hypercoagulability. Applied alone, luzindole increases significantly free TFPI (Fig. 2) ($p<0.01$), as well as its activity (Fig. 3) ($p<0.001$). This fact shows that melatonin receptors (MT_1 and MT_2) have multidirectional effects. On the one hand, they mediate the effects of melatonin, and on the other – their blocking activates the formation of both the free form TFPI, and free TFPI with high activity. Our study does not allow to conclude whether blocking of these receptors initiates increased biosynthesis of TFPI, or changes only in the surveyed indicators – free TFPI and free TFPI activity. Pretreatment with luzindole almost entirely repeats the results from the third group (luzindole only) and is a new evidence that the effects of exogenous and endogenous melatonin are blocked, and also for the significance of its receptors on the plasma level of free TFPI and its activity.

The presented in Fig. 4 and Fig. 5 results show the effects of melatonin and luzindole on vWF, defined as antigen and activity. The interpretation of these results would have been accurate in the light of the literature data which show that vWF, on the one hand, has a role in triggering normal hemostasis, and the other – the increase of its plasma level is to be regarded as a risk thrombogenic factor (16, 23). Therefore, the significantly elevated vWF antigen level (Fig. 4) ($p<0.001$) and its activity (Fig. 5) ($p<0.01$) found in this case, is accepted as a fact, which allows to say that melatonin induces tendency to hypercoagulability in rats. No less important information give the results obtained from the group of rats injected with luzindole. In this case, the blocking of melatonin receptors (MT_1 and MT_2) induces a strong decrease of both the vWF antigen level (Fig. 4) ($p<0.001$), and vWF ac-

tivity (Fig. 5) ($p<0.001$). We believe that the significantly reduced values were primarily the result of the inability of endogenous melatonin to achieve its stimulating effects on the formation of vWF and its activation. From the application of luzindole followed by melatonin (the fourth group of rats) can be concluded that in essence it is not different from the just mentioned results, i.e. the third group (luzindole only). It is imperative to add that in this case, the blocking of melatonin receptors blocks the effects of both endogenous and exogenous melatonin, which may explain the significantly lower values of vWF:Ag (Fig. 4) ($p<0.001$) and vWF:Act (Fig. 5) ($p<0.001$) than those of the control group. It is seen that melatonin is an important regulator of the whole chain – from the biosynthesis of vWF to the formation of high activity vWF.

These results not only confirm the established in our previous studies effect of melatonin on hemostasis, namely – a tendency to hypercoagulability (22,24,25), but also indicate that this hormone causes significant changes in TF, TFPI and vWF, which are the basis of this trend.

CONCLUSION

The results of our study allow us to summarize that the pineal hormone melatonin induces a pronounced tendency to hypercoagulability in rats by a significant increase of TF, decrease in plasma levels of free TFPI and free TFPI activity, as well as by an increase in vWF antigen and vWF activity. Luzindole self-administration and luzindole pretreatment show decisive involvement of MT_1 and MT_2 receptors for accomplishing the effects of the hormone.

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