Effects of Melatonin on The Tissue Factor Activities of Kidney, Heart and Brain in Experimental Renovascular Hypertension

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ÖZET
Deneysel renovasküler hipertansiyonda melatoninun böbrek, kalp ve beyin doku faktörü aktiviteleri üzerine etkileri

Amaç: Çalışmamızın amacı anjiotensin II bağımlı renovasküler hipertansiyon (RHV) oluşturulmuş sıçanların böbrek, kalp ve beyin dokularının doku faktörü aktivitesi (TFA) üzerine melatoninun etkilerini inclemektir.

Yöntem: RHV Wistar albino sıçanlarında renal arter klips (iki böbrek, bir klips, 2K1C) yerleştirerek oluşturulmuş, kontrol grubuna ise klips yerleştirilmemiştir. Operasyon gününde (RHV-Mel-1) veya operasyondan 3 hafta sonra başlayacak şekilde (RHV-Mel-2) sıçanlara 6 hafta boyunca melatonin (10 mg/kg/gün), kontrol grubuna ise 1 mL/kg/gün salin ile %1 alkol verilmiştir. İndirekt kan basıncı (BP) ölçümü “tail-cuff” metodu ile yapılmıştır. 9. haftanın sonunda BP ölçümlerini yapmıştır. Böbrek, kalp ve beyin dokuları çıkarılıp TFA ölçümüne kadar -20°Cde saklanmıştır. Dokuların TF aktiviteleri “Quick’s one stage” metoduyla göre yapıldı ve sonuçlar saniye olarak verildi.

Bulgular: 2K1C modeli BP'de ve böbrek TFA'da kontrolle karşılaştırıldığında anlamlı artışa neden oldu; ancak, kalp ve beyin TFA'yı etkilemedi. RHV-Mel-1 grubunda, böbrek TFa değerleri, RHV grubundan azaldı. Böbrek TFa değerlerinin RHV-Mel-2 grubunda kontrol grubuna göre artmış olduğu bulundu. Ancak kalp TFa değerleri kontrol grubunda artışlıydi melatoninun ile tedavi edilen her iki RHV grubunda arttı.

Sonuç: RHV grubunda artmış böbrek TFa renal arter stenozu ve tromboz gelişimine neden olabilir. Melatoninun ile tedavi edilen gruplarda artmış kalp TFa değerleri ise bu hayati organı aşırı kanamadan koruyacak ilave bir hemostatik koruma sağlayabilir.

Anahtar sözcükler: Renovasküler hipertansiyon, melatonin, doku faktörü, böbrek, kalp, beyin, sıçan

ABS TRACT
Effects of melatonin on the tissue factor activities of kidney, heart and brain in experimental renovascular hypertension

Objective: The aim of the present study was to investigate the effect of melatonin on tissue factor activities (TFA) of kidney, heart and brain in an angiotensin II-dependent renovascular hypertension (RVH) in rats.

Methods: RVH was induced in Wistar albino rats by placing renal artery clip (two-kidney, one-clip; 2K1C). Starting on the operation day (RHV-Mel-1) and 3 weeks after the operation (RHV-Mel-2), the rats received melatonin (10 mg/kg/day) while the control group received 1 mL/kg/day saline with 1% alcohol for the following 6 weeks. Indirect blood pressure (BP) measurement was made by the tail-cuff method. At the end of the 9th week, after BP recordings, kidney, heart and brain tissues were excised and stored at -20°C for the analysis of TFA. TFA of tissues were determined according to Quicks stage one method and results are expressed as seconds.

Results: 2K1C caused increase in BP and TFA of the kidney significantly but did not change TFA of heart and brain compared with the control group. In RVH-Mel-1 group, TFA of kidney decreased compared with the RVH group. TFA of kidney increased in RVH-Mel-2 group compared with the control. On the other hand, the TFA of heart increased in both melatonin-treated RVH groups compared with the control group.

Conclusion: Increased TFA of kidney in RVH group might promote renal artery stenosis and thrombosis development. On the other hand, increased TFA of heart in melatonin treated groups might provide additional hemostatic protections to this vital organ in order to prevent from excessive bleeding.

Key words: Renovascular hypertension, melatonin, tissue factor, kidney, heart, brain, rat

INTRODUCTION

Renovascular hypertension (RHV) caused by renal artery stenosis (RAS) leads to stimulation of the renin–angiotensin system which concomitantly increases production of angiotensin II (Ang II). RVH is most commonly caused by atherosclerotic renovascular disease although most patients do not have a significant atherosclerotic RAS. Other risk factors include age, male gender, smoking and a history of hypertension, diabetes mellitus or hyperlipidaemia.
Oxidative stress has been implicated causatively in the pathophysiology of many cardiovascular conditions, including hypertension (2). Ang II is a potent mediator of oxidative stress and a positive feedback mechanism could be established in the vessel wall for oxidative stress, endothelial dysfunction and inflammation through Ang II activation. Notably, Ang II can also upset the balance between the fibrinolytic and coagulation systems via its effect on vascular cells (3).

TF (Thromboplastin, Factor III) is a transmembrane 45-kDa protein constitutively expressed on the cells throughout the body and represents a key player in regulating the haemostatic and thrombotic response to injury (4). Various tissues and body fluids have been reported to have TF activity (TFa) (5-10). TF comes into contact with blood on disruption of the arterial wall integrity or if vascular cells express it on their surface (11). TF acts as the primary link between vascular cells and the haemostatic system by binding factor VII/VIIa with high affinity, resulting in the activation of the extrinsic pathway of the blood coagulation cascade (12). The TF/VIIa complex promotes the activation of factor IX and factor X with subsequent thrombin formation. Thrombin, generated in abundance at sites of vascular injury, plays a central role in the pathogenesis of atherosclerotic and thrombotic diseases. Moreover, thrombin not only catalyzes the conversion of fibrinogen to fibrin, thus triggering rapid fibrin deposition and clot formation, but also induces the expression of different chemotactic and procoagulant factors in vascular cells (13). Ang II may activate the coagulation cascade by increasing TF expression in vascular endothelial cells or thrombin activation (3,14).

Melatonin is produced in the central nervous system, specifically in the pineal gland (15), however its production is by no means limited to this organ. It has recently been discovered that it can be found in many tissues of the body, e.g. the placenta, kidneys, the respiratory tract, ventricles, the digestive system (stomach, liver, gall bladder, intestines), retina, some bone marrow cells, peripheral lymphocytes, skin, and possibly in many other cells as well (16-19). Besides its endogenous production, it is also ingested in the diet since melatonin is present in plants including edible foodstuffs. Consumption of foodstuffs containing melatonin is followed by its absorption into the blood (20). Melatonin has been shown to be involved in the regulation of many physiological systems including cardiovascular system (21). Melatonin reduces blood pressure (22) and has an antiadrenergic action on myocardial contractility (23). These effects are mediated by its receptors in the heart (24) and arteries (25). On the other hand, acting directly as an electron donor, melatonin scavenges free radicals, stimulates antioxidant enzyme systems (26) and along with its metabolites it has powerful anti-inflammatory properties proven to be highly effective in oxidative stress and inflammation (27). Sener et al. (28) showed that treatment of 2K1C rats with the antioxidant melatonin improved the cardiovascular dysfunction, reversed the asymmetric dimethylarginine levels and ameliorated the oxidative tissue damage. However, TFa of RHV induced rat tissues has not been investigated before. Consequently, the aim of this study was to investigate whether exogenous melatonin affects the TFa of brain, kidney and heart in angiotensin II-dependent RVH model.

**MATERIALS AND METHODS**

**Animals**

All experimental protocols were approved by the Marmara University Animal Care and Use Committee. Male Wistar albino rats (n=30) (200-250 g) were kept at a constant temperature (22±1°C) with 12 h light and dark cycles and fed with a standard rat chow.

**Surgery and Experimental Design**

Two-kidney, one-clip (2K1C) has been studied as an Ang II-dependent model of RVH with elevated circulating levels of Ang II and high Ang II concentration in the cortical tissue of the clipped and non-clipped kidneys (29). RHV was induced by clipping of the left renal artery as previously described (30). Briefly, a silver clip (internal diameter 0.25 mm) was placed around the left renal artery (2K1C group; n=24) of the rats that were anesthetized with ketamine (100 mg/kg) and chlorpromazine (0.75 mg/kg) given intraperitoneally (i.p.). Starting on the day of surgery, rats received melatonin (10 mg/kg/day; i.p.) for 9 weeks (early treatment) (RHV-Mel-1), while in a subgroup of 2K1C rats melatonin treatment was started at the end of the 3rd week following the surgery and continued for the remaining 6
weeks (late treatment) (RHV-Mel-2). The control group received 1 mL/kg/day saline with 1% alcohol. In order to obtain the basal values, blood pressure (BP) recordings were obtained in all rats before the surgical procedures, and these measurements were repeated at the end of the 3rd and 9th weeks after the surgeries. At the end of the 9th week, echocardiographic measurements were done in all rats before they were decapitated. Kidney, heart and brain tissues were excised and rinsed from blood in isotonic saline, blotted dry, weighed and stored at -20°C until the analysis of TF activity.

Determination of Tissue Factor Activity

The tissues were homogenized in 0.9% NaCl to obtain 10% tissue homogenates. The homogenates prepared from the tissues were used to determine the TFa. The TFa of the tissues were measured by Quick’s one-stage prothrombin time determination test (31). In one-stage prothrombin time determination test, a commercial tissue thromboplastin is mixed with equal amounts of plasma and Ca++. A firm clot forms in a short time, the time (sec) being dependent on the potency of the TF used. Since the clotting time is inversely proportional to the TFa, the lengthening of the clotting time is a manifestation of decreased TFa.

Statistics

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego; CA; USA). All data were expressed as means ± SD. Groups of data were compared with Kruskal Wallis test followed by Dunn’s multiple comparison tests. Values of p<0.05 were regarded as significant.

RESULTS

The basal blood pressures recorded before the surgery were not different among the groups. In the vehicle-treated group with 2K1C, the blood pressure was significantly elevated at the third- (172±6.2 mmHg; p<0.001) and ninth- (190±9.3 mmHg; p<0.001) week recordings with respect to basal values (124±8.4 mmHg). Similarly, at the 3rd week measurement of the 2K1C, late treatment group (RHV-Mel-2) that has not received melatonin yet, blood pressure was elevated (169±7.5 mmHg; p<0.001) to the levels of vehicle-treated 2K1C group. However, at the 9th week measurement of RHV-Mel-2 group, the blood pressure was reduced significantly (144±6.2 mmHg; p<0.001), but was still higher with respect to basal values (p<0.05). In the 2K1C group with early melatonin treatment (RHV-Mel1), blood pressure was not elevated either at the 3rd or 9th week measurements, indicating that melatonin treatment abolished 2K1C-induced hypertension.

TFa of kidney, heart and brain in RHV, RHV-Mel1, RHV-Mel2 and the control groups were 25.33±2.35, 28.82±3.76, 22.85±2.13, and 24.21±2.46 sec, respectively. The results of the statistical analysis are presented in Figures 1 and 2.

Figure 1: Tissue Factor Activities of kidney in experimental renovascular hypertension (RHV) group, in melatonin treated RHV group starting either on the operation day (RHV-Mel 1) or 3 weeks after the operation day (RHV-Mel 2), and the control group.

Figure 2: Tissue Factor Activities of heart in experimental renovascular hypertension (RHV) group, in melatonin treated RHV group starting either on the operation day (RHV-Mel 1) or 3 weeks after the operation day (RHV-Mel 2), and the control group.
27.80±1.3, 30.80±1.48; 51.75±8.1, 39.38±3.82, 42.80±1.92, 54.80±4.6 and 40.67±6.0, 41.89±5.58, 38.2±5.54, 49.0±7.18 sec, respectively. Experimentally induced RHV increased the TFa of kidney significantly but did not change TFa of heart and brain in the RHV group compared with the control group (Fig. 1, 2 and 3). In RHV group, melatonin treatment which was started on the operation day decreased the TFa of kidney compared with the RHV group (Fig. 1). On the other hand, the TFa of heart was increased in both melatonin-treated RHV groups when compared with the saline treated RHV and the control groups (Fig. 2).

DISCUSSION

The renin-angiotensin system is crucial for the maintenance of blood pressure, fluid, and sodium homeostasis and thus, plays a major role in the pathogenesis of hypertension. RHV, a relatively rare form of secondary hypertension, is associated with the activation of renin-angiotensin system as a result of reduced renal blood flow and perfusion pressure due to renal artery stenosis (32). The immediate hypertensive effects of Ang II occur as a result of vasoconstriction and antiatriuresis. However, in the long-term, Ang II causes remodeling of the arterial vasculature by vascular remodeling, accelerated atherogenesis, extracellular matrix deposition and glomerulosclerosis, all of which may contribute to the progression of cardiovascular and renal damage beyond the effects of high blood pressure alone (33,34).

In the present study, we evaluated TFa of kidney, heart and brain in an Ang II-dependent RVH model and also investigated the effects of melatonin on these activities. RHV increased the TFa of kidney significantly but did not change TFa of heart and brain in the RHV group compared with the control group. TF plays an important role in cardiovascular diseases in vasculopathies, reperfusion injury, preeclampsia, and kidney disease but also has biological functions independent of the clotting cascade (35,36). Ang II has been shown to stimulate TF (37). Moreover, Ang II was shown to be a powerful stimulator of endothelin 1 (ET-1) synthesis and release in vascular smooth muscle and endothelial cells (38). Furthermore, Ang II-induced tissue ET-1 fosters vascular hypertrophy (39). Ang II can activate the nuclear transcription factor NF-κB. NF-κB is primarily responsible for the transcription of monocyte chemoattractant protein-1 (MCP-1) and adhesion molecules leading to inflammation (40). NF-κB also regulates the transcription of TF. Constitutive TF expression by mesenchymal cells in the adventitial blood vessel lining normally precludes TF interaction with factor VII in plasma but allows activation of coagulation when the endothelium is damaged (41). Accordingly in the present study, increased TFa of kidney in RHV group might promote renal artery stenosis and thrombosis development.

In RHV group, melatonin treatment which was started on the operation day decreased the TFa of kidney compared with the RHV group. Melatonin is a strong antioxidant (26,27). It directly sweeps away free oxygen radicals and eliminates them indirectly through stimulation of antioxidative enzymes (26,27). It limits the generation of free radicals formed during the Fenton’s reaction by chelating metal ions of transition groups (Cu²⁺, Fe²⁺), therefore melatonin limits lipid, protein and DNA peroxidation process. It limits pathological states developed on the basis of oxidative stress, i.e., neurodegenerative diseases (e.g. Alzheimer’s disease or tumors) (26,27). Ferro et al. (42) suggested that oxidative stress contributes to activate the clotting system via over-expression of monocyte TF and showed a close relationship between increased lipid peroxidation and monocyte TF overexpression. The role of oxidative stress in enhancing monocyte TF was further corroborated by in vivo study showing that antioxidant treatment significantly reduced monocyte TF expression. Antioxidant treatment has also
been shown to reduce monocyte TF in patients with liver cirrhosis (43). Therefore, decreased TFa of kidney may be due to decreased oxidative stress in melatonin treated rats.

On the other hand, the TFa of heart was increased in both melatonin-treated RHV groups when compared with the control group. Increased TFa of heart might provide additional hemostatic protections to this vital organ in order to prevent from excessive bleeding.

In this study increased TFa of kidney in RHV group might promote renal artery stenosis and thrombosis development. Melatonin treatment decreased the TFa of kidney which may be due to decreased oxidative stress in melatonin treated rats. On the other hand, increased TFa of heart in both melatonin-treated RHV groups needs further investigation.

REFERENCES


