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RESEARCH ARTICLE

The Effect of Melatonin on Some Coagulation Parameters in Streptozotocin-induced Diabetic Rats

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ABSTRACT

The aim of this study is to evaluate the possible protective effects of melatonin on hemostatic parameters in diabetic rats. For this purpose, 32 adult, male, healthy Wistar Abino rats were seperated into four groups. Control group didn't exposure any trial. Melatonin group was treated with 50 mg/kg melatonin by intraperitoneally during 8 weeks. In diabetes group, diabetes was induced by subcutaneous injections of streptozotocin at dose of 40 mg/kg for two days as a single daily dose. Diabetes+Melatonin group was consist of the animals that treated with 50 mg/kg melatonin by intraperitoneally to streptozotocin induced diabetic during 8 weeks. In diabetic rats, the platelet count and fibrinogen level significantly increased compared to control group ($p<0.05$), whereas melatonin application to the diabetic rats caused to decrease in fibrinogen level when compared to diabetic rats ($p<0.05$). Activated Partial Tromboplastin Time (APTT), Prothrombin Time (PT) and International Normalized Ratio (INR) levels significantly shortened in the experimentally diabetes group compared to the control group ($p<0.05$). PT and INR significantly prolonged in diabetic rats with the melatonin treatment compared to diabetic rats ($p<0.05$). In conclusion, the obtained data indicated that administration of melatonin partly ameliorated procoagulant state caused by diabetes in rats.

Keywords: APTT, fibrinogen, diabetes, melatonin, rat

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Streptozotosin ile Diyabet Oluşturulan Ratlarda Melatonin Uygulamasının Bazı Koagulasyon Parametreleri Üzerindeki Etkisi

ÖZ

Bu çalışmanın amacı streptozotosin ile diyabet oluşturulan ratlarda hemostatik parametreler üzerine melatoninin muhtemel koruyucu etkilerinin belirlenmesidir. Bu amaçla 32 yetişkin erkek sağlıklı Wistar Abino rat dört gruba ayrıldı. Kontrol grubuna herhangi bir uygulama yapılmadı. Melatonin grubuna 8 hafta boyunca intraperitoneal olarak 50 mg/kg melatonin uygulandı. Diyabet grubunda 40 mg/kg streptozotosinin günlük tek doz olmak üzere iki gün subkutan enjeksiyonuyla diyabet oluşturuldu. Diyabet+Melatonin grubuna streptozotosin uygulanarak diyabet oluşturulduktan sonra 8 hafta boyunca intraperitoneal olarak 50 mg/kg melatonin enjekte edildi. Diyabetik ratlarda platelet sayısı ve fibrinojen seviyesi kontrol grubuna göre önemli oranda artarken ($p<0.05$), diyabetik ratlara melatonin uygulaması diyabet grubuna göre fibrinojen düzeyinde azalmaya neden oldu ($p<0.05$). Deneysel diyabet grubundaki APTT, PT ve INR düzeyleri kontrol grubuna göre önemli bir şekilde azaldı ($p<0.05$). Diyabetik ratlara intraperitoneal olarak melatonin uygulaması ile PT ve INR düzeyleri diyabetik ratlara göre önemli bir şekilde uzadı ($p<0.05$). Sonuç olarak, bu çalışmadan elde edilen veriler melatonin uygulamasının ratlarda diyabetten kaynaklanan prokoagulan durumu kısmen düzelttiğini göstermektedir.

Anahtar Kelimeler: APTT, fibrinojen, diyabet, melatonin, rat

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INTRODUCTION

In the worldwide, there is an increase in death due to atherothrombotic disorders result from metabolic disorders such as diabetes mellitus (DM), hypertension and obesity (Sanz and Fuster 2011, Korish et al. 2015). Diabetes mellitus caused by either low insulin level or high insulin resistance is characterized with high hyperglycemia and the other metabolic disorders (Yeom et al. 2016). Millions of people are affected by cardiovascular diseases related to diabetes mellitus (Kakouros et al. 2011). In later stages, diabetes mellitus causes abnormal endothelial function, increase of arterial stiffness, platelet hyperreactivity and hemorheological changes (Wilkinson et al. 2000, Schäfer et al. 2007, Cho et al. 2008, Yeom et al. 2016). Another complication of diabetes is hemostatic disorders. Some studies claim that diabetes increases the tendency to coagulation (Takada et al. 1993, Osende et al. 2001, Creager et al. 2003, Ferreiro et al. 2010, Ferreiro and Angiolillo 2011). These serious disturbances play a crucial role in the etiology of diabetes-related vascular complications including arteriosclerosis and myocardial infarction (Beckman et al. 2002, Loomans et al. 2004, Yeom et al. 2016). Thrombotic disorders and vascular lesions such as microvasculopathy, retinopathy and macroangiopathy, especially in the coronary and cerebral vessels can be life threatening. Rupture of an atherosclerotic plaque promotes platelet activation and locally triggering of the coagulation process that can cause thrombus formation at the region of endothelial damage (Kakouros et al. 2011). It has been stated that cardiovascular and cerebrovascular disorders frequently occurred in diabetes due to micro- and macrovascular complications (American Diabetic Association 2011, Eriksson et al. 2012, Korish et al. 2015).

Many therapeutic approaches have been still studied to alleviate the complications of diabetes. Recently, studies have been focused on melatonin as a therapeutic agent due to its several physiological activities (Carrillo-Vico et al. 2005, Claustrat et al. 2005). In 1958, Lerner's group first isolated melatonin (N-acetyl-5-methoxy-tryptamine) from the bovine pineal gland (Minneman and Wurtman 1976). Melatonin production is regulated by the suprachiasmatic nucleus (SCN) as known the central circadian pacemaker (Carrillo-Vico et al. 2005). It is synthesized in several organs, including the pineal gland, Harder's glands, gastroenteric mucous membrane, retina, platelets and megakaryocytes (Reiter et al. 1988). Melatonin has high lipid and water solubility features, thus it passes easily across cell membranes (Claustrat et al. 2005). Pineal secretion reaches maximum plasma levels around 03:00–04:00 a.m. During the day, its level is low or even undetectable (Follenius et al. 1995, Rodella et al. 2013).

Although, there is a limited data about the effects of melatonin on coagulation, there are various findings about the effect of melatonin on cardiovascular incidents, hemorrhage, activities of some coagulation proteins and fibrinolytic systems regarding in subjected to circadian variations (Pinotti et al. 2005, Montagnana et al. 2009). Some studies reported that melatonin may used for hemostasis bu using in a different route, doses and period to animals and human (Tunali et al. 2005, Tai et al. 2010, Kostovski et al. 2011). It was also suggested that there is a dose-dependent relationship between plasma melatonin level and coagulation activity (Wirtz et al. 2008, Pashalieva et al. 2014).

Based upon these acknowledgements we aimed to determine the effects of melatonin on some coagulation parameters in streptozotocin-induced diabetic rats.

MATERIALS and METHODS

In the study, 32 male, 6 weeks of age, healthy Wistar Abino rats were used. The animals were divided into four groups. All animals were fed with standart rat diet as *ad libitum* during 8 weeks. The Ethical Committee of Selcuk University Experimental Medicine Research and Application Center approved the study protocol (Report no. 2017-15).

- The animals in control group (n=6) didn't exposure any treatment.
- Melatonin group (n=6) was injected with 50 mg/kg melatonin (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally during 8 weeks.
- Diabetes group (n=10) was induced by subcutaneously injected with streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) at dose of 40 mg/kg in 0.1 M citrate buffer (pH 4.5) for two days as a single daily dose.
- Diabetes+Melatonin group (n=10) was injected with 50 mg/kg melatonin intraperitoneally to the diabetic rats during 8 weeks.

In the cases of streptozotocin-induced hypoglycemia, rats were given 5% dextrose solution as a precaution after 6 h of streptozotocin administration during next 3 days. Diabetes was verified by measuring blood glucose level strips using glucometer (PlusMED Accuro, Taiwan) via the tail vein after one week from streptozotocin injections. Animals, which have a blood glucose level higher than 250 mg/dl, were accepted as diabetic and were included in the experiment. During the experiment, one animal from diabetes group were died due to streptozotocin-induced hypoglycemia.

At the end of the 8 weeks, blood samples were taken from all animals. In these blood samples, platelet, fibrinogen, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR) levels were determined. Platelet, fibrinogen, APTT, PT, INR levels were determined by using Abbott kits in Abbott analyzer (Abbott Architect i2000).

The data obtained from the study were analyzed by one-way ANOVA (SPSS 19). Differences among the groups were determined by Duncan's multiple range test. Differences were considered significant at $p<0.05$.

RESULTS

The effect of melatonin on coagulation parameters in experimentally induced diabetes in rats were summarized Table 1. In diabetic rats, the platelet count and fibrinogen level significantly increased compared to control group ($p<0.05$, Table 1), whereas intraperitoneally melatonin application to the diabetic rats caused to decrease in both parameters when compared to diabetic rats. The decrease in fibrinogen level was significant ($p<0.05$, Table 1). In this study, APTT, PT and INR levels in the experimentally diabetes group significantly shortened compared to control group ($p<0.05$, Table 1). With the intraperitoneally melatonin application to the diabetic rats, PT and INR significantly prolonged compared to diabetic rats ($p<0.05$, Table 1), while the changes in APTT was not important statistically.

DISCUSSION

In diabetes mellitus, cardiovascular complications and the other metabolic disorders generally coexist and lead to high morbidity and mortality in worldwide. It is frequently seen that prothrombotic conditions such as platelet hyperreactivity, impaired fibrinolysis, endothelial dysfunction and increased coagulation cause these cardiovascular complications in diabetes. (Takada et al. 1993, Osende et al. 2001, Creager et al. 2003). Some mechanisms caused by metabolic and cellular abnormalities have been accepted as a reason of coagulation tendency in diabetes mellitus. These mechanisms and their ethiologies were been categorized as hyperglycemia, insulin deficiency and resistance associated with metabolic conditions and other cellular abnormalities. (Ferreiro et al. 2010, Ferreiro and Angiolillo 2011). In parallel with the knowledge that mentioned above, coagulation parameters were significantly impaired in streptozosin induced diabetic rats in this study. So that, thrombocyte count and fibrinogen level significantly increased in diabetic group compared to control group ($p<0.05$, Table 1), whereas APTT, PT and INR shortened compared to control group ($p<0.05$, Table 1). Ohaeri and Adoga (2006) reported that the increases in platelet count and coagulation factors amount (factor V, VII, VIII, IX and X) were determined in experimentally diabetic rats compared to control rats. In addition, plasma fibrinogen levels were found to be higher as parallel to hyperglycemic degree in many studies conducted with diabetes (Ceriello et al. 1994, Schalkwijk et al. 1999, Dunn and Ariëns 2004). In another study, it has been stated that PT, APTT and coagulation time in diabetic rats were found to be lower than control group, while platelet count and plasma fibrinogen levels in diabetic rats were obtained as higher than control group's level (ElGendy and Abbas 2014). It was suggested that hyperglycemia exposure may induce procoagulant state in diabetes due to hemorheological changes of thrombocytes, platelet aggregation, endothelial dysfunction and increasing levels of tissue factors (Yeom et al. 2016). Another pathway of activation of procoagulant state is that hyperglycemia leads to increasing levels of tissue factor, prothrombin fragments, decreasing factor VII/VIIa and increasing factor VIII (Vaidyula et al. 2006b). In addition, it was reported that hyperglycemia upregulate platelet expression of CD40L and increase monocyte-platelet aggregates as indicative of platelet activation (Vaidyula et al. 2006a, Kakouros et al. 2011).

Melatonin, a signaling molecule for circadian rhythms, plays an important role in many biological processes (Dahm et al. 2006, Kostovski et al. 2011). It was suggested that endogenous melatonin may also produce antithrombotic, antioxidant and anti-inflammatory activity (Carrillo-Vico et al. 2005, Claustrat et al. 2005, Dahm et al. 2006, Ashy and Shroff 2016). Depend on its wide effects, melatonin could be expected to regulate hemostatic events and the effects of melatonin on hemostasis are relatively poorly studied (Kostovski et al. 2011). For this reason, we determined some hemostatic parameters in diabetic rats treated with melatonin. Melatonin treatment significantly decreased plasma fibrinogen levels compared to diabetic group, while PT and INR levels significantly increased when compared diabetic group ($p<0.05$, Table 1). The changes in platelet count and APTT with the melatonin treatment to diabetic rats were not important statistically. In the light of our results, it might be said that melatonin has favorable effects on hemostatic parameters in streptozotocin-induced diabetic rats. These effects of melatonin on hemostasis such as inhibiting procoagulant state were attributed to some mechanisms. Kostovski et al. (2011) reported that melatonin increased tissue factor pathway inhibitor (TFPI) in endothelial cells *in vitro*. In contrary, it was suggested that plasma TFPI level is low when plasma melatonin levels peak in darkness, vice versa (Dahm et al. 2006). Protein C anticoagulant pathway is one of important mechanisms of the anticoagulant system. This pathway includes membrane proteins and circulating proteins (such as protein C, thrombomodulin, endothelial protein C receptor

(EPCR) and protein S) (Castellino and Ploplis 2009, Stancheva et al. 2015). Castellino and Ploplis (2009) stated that melatonin significantly reduced activity of protein C anticoagulant pathway in rats. The beneficial effects of melatonin related to procoagulant state might based on endothelium protective effects (Rodella et al. 2013). In diabetes, increased in plasma glucose levels contribute endothelial dysfunction characterized by proliferating barrier function and adhesion of circulating cells (Ho et al. 2000, Ido et al. 2002, Favaro et al. 2008). Therefore, melatonin with its antioxidant and antiinflamatory properties is being expected to act protective on endothelial dysfunction arise from diabetes. Thus, melatonin activates antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase (Rezzani et al. 2006, Bharti and Srivastava 2009), protects cells from oxidative damage and apoptosis (Jou et al. 2007, Rodella et al. 2013).

Table 1. Effect of melatonin on coagulation parameters in diabetes (Mean±SE)

	Platelet (K/μl)	Fibrinogen (mg/dl)	APTT (sec)	PT (sec)	INR
Control	690.50±27.65^{b}	227.83±22.39^{b}	35.62±3.30^{a}	11.60±0.21^{ab}	1.05±0.02^{a}
Melatonin	711.83±37.62^{b}	193.67±18.79^{b}	36.78±3.18^{a}	11.80±0.10^{a}	1.06±0.01^{a}
Diabetes	827.56±20.71^{a}	313.56±24.37^{a}	24.21±2.80^{b}	9.79±0.29^{c}	0.90±0.02^{b}
Diabetes+Melatonin	747.20±25.44^{ab}	248.30±18.71^{b}	31.59±2.14^{ab}	10.92±0.24^{b}	1.00±0.02^{a}

$^{a-c}$ The difference between mean values with different superscripts in the same column is significant at the $p<0.05$ level.

CONCLUSIONS

As a result, the obtained data from this study indicated that administration of melatonin partly improved procoagulant state caused by diabetes in rats. Nevertheless, the further studies are needed established with different melatonin doses related to hemostatic state in diabetes in the future.

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