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RESEARCH ARTICLE

The effect of melatonin on coagulation parameters in rats with cerulein-induced acute pancreatitis

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Serulein ile akut pankreatit oluşturulan ratlarda melatoninin koagulasyon parametreleri üzerine etkileri

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Öz

Amaç: Bu çalışmanın amacı cerulein ile akut pankreatit oluşturulan ratlarda melatoninin koagulasyon parametreleri üzerine olası etkilerini değerlendirmektir.

Gereç ve Yöntem: Bu amaçla 32 yetişkin erkek sağlıklı Wistar Abino rat kullanıldı. Hayvanlar dört gruba ayrıldı. Grup 1'deki hayvanlara herhangi bir uygulama yapılmadı. Grup II'deki hayvanlara iki saat aralıkla iki kez 50 mg/kg melatonin intraperitoneal olarak uygulandı. Grup III'deki hayvanlara iki saat aralıkla iki intraperitoneal serulein (sırasıyla, 50 µg/kg ve 25 µg/kg bw) enjeksiyonu yapıldı. Grup IV'deki hayvanlara iki saat aralıkla iki intraperitoneal serulein (sırasıyla, 50 µg/kg ve 25 µg/kg bw) enjeksiyonu yapıldı ve ratlara her bir serulein enjeksiyonundan 30 dk önce intraperitoneal olarak 50 mg/kg melatonin enjeksiyonu yapıldı. Hayvanlardan alınan kan örneklerinde platelet, fibrinojen, APTT, PT, INR düzeyleri belirlendi.

Bulgular: Platelet sayısı ve fibrinojen seviyesi akut pankreatite bağlı olarak kontrol grubu ile karşılaştırıldığında arttı ($p<0.05$). Akut pankreatit grubu APTT, PT ve INR düzeyleri kontrol grup düzeyi ile karşılaştırıldığında önemli bir şekilde kısaldı ($p<0.05$). Akut pankreatitli ratlara melatonin uygulaması ile PT ve INR düzeyleri akut pankreatitli grup ile karşılaştırıldığında önemli oranda uzadı ($p<0.05$).

Öneri: Çalışmada, melatoninin akut pankreatite bağlı olarak gelişen koagulasyon parametrelerindeki olumsuz değişiklikleri hafifletebileceği sonucuna varıldı.

Anahtar kelimeler: Serulein, melatonin, akut pankreatit, koagulasyon parametreleri, rat

Abstract

Aim: The aim of this study was to evaluate possible effects of melatonin on coagulation parameters in rats with cerulein induced acute pancreatitis.

Materials and Methods: For this purpose, 32 adult, male, healthy Wistar Abino rats were used. The animals were divided into four groups. Group I animals was no applied. Group II animals was intraperitoneally administered 50 mg/kg melatonin per rat twice for two hours intervals. Animals of group III received two intraperitoneal injections of cerulein (50 µg/kg and 25 µg/kg bw, respectively) at two hours intervals. Animals of group IV received two intraperitoneal injections of cerulein (50 µg/kg and 25 µg/kg bw, respectively) at two hours intervals and the rats received an intraperitoneal injection of 50 mg/kg melatonin 30 min before each cerulein injection. In blood samples taken from all animals, platelet, fibrinogen, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR) levels were determined.

Results: Acute pancreatitis caused increment in platelet count and fibrinogen level compared to control group ($p<0.05$). APTT, PT and INR were significantly shortened with acute pancreatitis in comparison with control group ($p<0.05$). Melatonin application to rats with acute pancreatitis importantly lengthened PT and INR compared to acute pancreatitis group ($p<0.05$).

Conclusion: We concluded that melatonin may be alleviated the abnormalities in coagulation parameters resulting from acute pancreatitis.

Keywords: Cerulein, melatonin, acute pancreatitis, coagulation parameters, rats

Introduction

Acute pancreatitis is a serious disease with an increasing incidence in human and animals. The rate of seeing of acute pancreatitis has increased two fold since 1960 (Goldacre and Roberts 2004, Baddeley et al. 2011). This disease, which is characterized by significant systemic inflammatory events, leads to varying degrees of organ dysfunction (Baddeley et al. 2011). The rate of mortality is 50% in patients with multi organ failure (Stevenson and Carter 2013). Pancreatitis is more common in cats and dogs. This disease in animals has similar processes and pathophysiology seen in human (Xenoulis et al. 2008). Coagulative disorders occurred in acute pancreatitis are related to its severity (Lasson and Ohlsson 1986, Salomone et al. 2003). The hemostatic system activated by acute pancreatitis causes formation of thrombi in blood vessels of this organ. These disorders have most events which range from scattered intravascular thrombosis to serious disseminated intravascular coagulation (Agarwal and Pitchumoni 1993). There are strictly relationship between inflammation and coagulation (Esmon et al. 1991). Inflammatory cytokines increases expression of tissue factor leading to formation of thrombi (Esmon 1999, Warzecha et al. 2007).

Antioxidant applications are important in terms of therapeutic approaches in order to prevent or alleviate acute pancreatitis and its complications caused by coagulation disorders. Melatonin is main secretory product of the pineal gland and is a powerful radical scavenger and antioxidant. It is accepted as more effective than either vitamins C or E (Bekyarova et al. 2010). Melatonin scavenges the reactive oxygen species (ROS) and nitrogen-based species (RNS). It also stimulates the activity of antioxidant enzymes and suppresses the expression of proinflammatory cytokines and adhesion molecules (Reiter 2000, Reiter et al. 2001, Rodriguez et al. 2004, Bekyarova et al. 2010).

The aim of this study was to evaluate possible effects of melatonin on coagulation parameters in rats with cerulein induced acute pancreatitis.

Materials and Methods

In the study, 32 adult (6 weeks), male, healthy Wistar Abino rats were used. The animals were divided into four groups. All animals were fasted before at the beginning of study, while it allowed to drink water.

Group I (C) (n=6): Group I animals was no applied.

Group II (M) (n=6): Group II animals was intraperitoneally administered 50 mg/kg melatonin (Sigma-Aldrich, St. Louis, MO, USA) per rat twice for two hours intervals.

Group III (AP) (n=10): Animals of group III received two intraperitoneal injections of cerulein (Sigma-Aldrich, St. Louis, MO, USA) (50 µg/kg and 25 µg/kg bw, respectively) at two hours intervals.

Group IV (APM) (n=10): Animals of group IV received two intraperitoneal injections of cerulein (50 µg/kg and 25 µg/kg bw, respectively) at two hours intervals and the rats received an intraperitoneal injection of 50 mg/kg melatonin 30 min before each cerulein injection.

After 12 hours from the last cerulein injection, 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 Architect i2000 analyzer.

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 acute pancreatitis were summarized in Table 1. In this study, acute pancreatitis caused increment in platelet count compared to control group ($p<0.05$, Table 1). Melatonin administration to rats with acute pancreatitis slightly decreased platelet count compared to group with acute pancreatitis and this level is also not different from control and melatonin groups. Fibrinogen level in acute pancreatitis group significantly increased as response to pancreatitis compared to control group ($p<0.05$, Table 1). In rats with acute pancreatitis, fibrinogen levels in the melatonin treated group were observed to be decreased close to the control group. APTT, as sign of coagulation activation, decreased in acute pancreatitis group compared with control group level ($p<0.05$, Table 1). Melatonin administration to rats with acute pancreatitis caused unimportantly increment in APTT when compared to acute pancreatitis group. PT and INR were significantly shortened with acute pancreatitis in comparison with control group ($p<0.05$, Table 1). Melatonin application to rats with acute pancreatitis importantly lengthened both parameters in comparison with acute pancreatitis group ($p<0.05$, Table 1).

Discussion

In the present study, abnormalities occurred in coagulation parameters such as platelet count, fibrinogen, APTT, PT and INR were considered to be related to severity of acute pancreatitis. In acute pancreatitis, several mechanisms are thought to be responsible for disorders in coagulation and hemostasis. First of them is cellular factors, which are represented by

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

	Platelet (K/µl)	Fibrinogen (mg/dl)	APTT (sec)	PT (sec)	INR
C	677.33±74.86^{b}	215.17±18.52^{b}	29.27±2.72^{a}	12.60±0.69^{a}	1.12±0.05^{a}
M	723.83±81.49^{ab}	212.67±16.43^{b}	31.53±4.38^{a}	12.98±0.12^{a}	1.16±0.01^{a}
AP	882.20±45.81^{a}	318.80±26.45^{a}	21.38±1.30^{b}	10.69±0.14^{b}	0.97±0.01^{b}
APM	765.50±46.09^{ab}	269.70±20.97^{ab}	27.04±2.17^{ab}	12.09±0.25^{a}	1.09±0.02^{a}

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

activated platelets. Second mechanism is humoral coagulator factors leading to fibrinogen activation as the final step of the coagulation cascade. The role of platelets in the process depends on their interactions with endothelium, leukocytes, and humoral coagulator and inflammatory proteins (Bouchard and Tracy 2001, Shebuski and Kilgore 2002, Hackert et al. 2007). Acute pancreatitis involves the infiltration of polymorphonuclear leukocytes, the accumulation of platelets intravascularly and extravascularly, as well as fibrin deposition in the connective tissue and intercellular spaces. All events above cause microthrombi in local blood vessels (Bockman et al. 1986, Kakafika et al. 2007). These microthrombi resulting in irreversible perfusion failure with consecutive tissue hypoxia and necrosis can trigger systemic inflammatory and septic complications (Wang et al. 1996, Hackert et al. 2007). In our study, the increases in platelet count and fibrinogen level and shortening in APTT, PT and INR represents coagulation activation depend on acute pancreatitis. These changes are consistent with above acknowledgement which were stated the changes in coagulation parameters caused by acute pancreatitis. Our present results also support that acute pancreatitis triggered blood coagulation via stimulation of the intrinsic and common pathways factors and interaction of inflammatory events.

Oxidative stress, chronic systemic inflammation and coagulation activation are accepted fundamental conditions to initiate and to progress of atherothrombotic disease such as coronary artery disease (Levi et al. 2004, Libby and Theroux 2005, Förstermann and Münzel 2006, Wirtz et al. 2008). Melatonin as a free radical scavenger, antioxidant, anti-inflammatuar, antiaggregatory is reported that it might be useful in suppression disorders of coagulation, procoagulant processes, inflammation and disseminated intravascular thrombosis (Bekyarova et al. 2010). It was reported that melatonin has pleiotropic action and could favorably influence the course of coronary artery disease (Carrillo-Vico et al. 2005, Claustrat et al. 2005, Dahm et al. 2006, Wirtz et al. 2008). Based on this acknowledgement, we used melatonin to investigate the effect on blood coagulation parameters in rats with cerulein induced acute pancreatitis. The treatment with two dose of 50 mg/kg melatonin at two hours intervals resulted in prolongation of PT and INR levels (p<0.05, Table 1), while striking changes were observed in platelet count, fibrinogen and APTT levels. The effects of melatonin on these coagulation parameters may be accepted positively and attributed anti-inflammatory and antiaggregatory properties. It is reported that there is functionally link between inflammatory cells and humoral coagulation factors (Hackert et al. 2007). In addition, melatonin inhibits the proinflamatuar cytokines such as TNF-α and IL-6 cytokines (Reiter et al. 2000, Bekyarova et al. 2010). These cytokines are released by activated leucocytes during systemic inflammatory process and contribute release of tissue factor triggering intravascular coagulation (Okajima 2000). C-reactive protein (CRP) as an acute phase protein also provokes procoagulant states (Dhainaut et al. 2001, Bekyarova et al. 2010). It was suggested that oral melatonin treatment inhibited platelet aggregation and reduced leukocyte rolling (Lotufo et al. 2001). Tunali et al. (2005) reported that melatonin normalized shortened prothrombin time and elevated levels of fibrin degradation products in rats. Wirtz et al. (2008) found that plasma FactorVIII and fibrinogen are lower in young men received melatonin than received placebo. It was suggested that melatonin could exert an indirect effect on plasma coagulation activities by its anti-inflammatory and antioxidative properties (Claustrat et al. 2005, Viles-Gonzalez et al. 2006, Muller et al. 2007, Wirtz et al. 2008).

Conclusions

Acute pancreatitis as depend on severity activates the coagulation pathways by stimulating several mechanisms such as platelet activation, inflammatory cytokine release and vessel wall or endothelial impairment. In the light of these results, melatonin, which has antioxidant and anti-inflammatuar properties, alleviated the abnormalities in coagulation parameters resulting from acute pancreatitis and melatonin might be useful in the prevention of coagulopathy and microthrombosis in acute pancreatitis. However, future studies are needed to evaluate the clinical importance of melatonin administration for coagulation activity.

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