A study on effect of melatonin in dyslipidemia caused by experimentally diabetes

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Abstract

In this study, it was aimed to determine the effects of melatonin application on lipid profile in streptozotocin-induced diabetic rats. Animals in control group were not any treatment. Melatonin group animals received 50 mg/kg melatonin intraperitoneally in daily for eight weeks. Diabetes was induced by subcutaneous injections of streptozotocin at dose of 40 mg/kg for two days as a single dose per day in diabetes group animals. Animals in Diabetes+Melatonin group were made diabetic by streptozotocin in the same way and then these animals received 50 mg/kg melatonin intraperitoneally in daily for eight weeks. In blood samples taken from all animals, TNF-α, triglyceride, total cholesterol, LDL and HDL were determined. In diabetes group, TNF-α level were high (p<0.05). The changes in TNF-α level with melatonin application to diabetic rats were important (p<0.05). In diabetic rats, triglyceride and LDL levels were found to be enhanced compared to control group (p<0.05), HDL level was significantly low (p<0.05), while the change in total cholesterol level was not important. With the melatonin application to diabetic rats, HDL level was found to be higher (p<0.05) and LDL level was lower than diabetes group (p<0.05). It was concluded that the administration of melatonin to streptozotocin-induced diabetic rats may have positive effects on lipid profile.

Key words: Diabetes, melatonin, TNF-α, lipid profile, rats.

INTRODUCTION

Diabetes has serious complications in worldwide and its incidence is an increasing in recent years. Cardiovascular disorders are one of the most important complications of diabetes. The incidence of cardiovascular disorders in diabetic patients is 3-4 times higher than those without diabetes. Several risk factors such as dyslipidemia, hypertension and smoking are thought to be associated with the prevalence of micro and macrovascular disorders in diabetes. It is accepted that diabetes itself is an independent risk factor for development of atherosclerosis cases. Oxidative stress is one of the potential mechanisms that mediate vascular disorders in diabetes. Free radicals and oxidative stress play an important role in atherogenesis as well as cause oxidation of low density lipoproteins. Oxidized LDL (Ox-LDL) is recognized not only by LDL receptors but also by the cleansing receptor pathway in macrophages, which results in foam cell formation resulting in irregular cholesterol accumulation (14, 33).

Blood lipids are mainly cholesterol, triglycerides, phospholipids and free fatty acids. Hyperlipidemia plays an important role in the development of many cardiovascular disorders such as atherosclerosis, angina pectoris, myocardial infarction and stroke (16). Excessive inflammatory fibroproliferative events in response to various conditions lead to atherosclerotic lesions by damaging the arterial wall smooth muscles and endothelium (23). Increased lipid accumulation in vascular endothelium is one of the factors that trigger inflammatory processes (16). It is suggested that increased C-reactive protein (CRP) and TNF-α due to hyperlipidemia are closely related to the onset of the inflammatory process and thus anti-inflammatory applications against hyperlipidemia may be effective (26). There are also reports that inflammatory events independent from hyperlipidemia are associated with the onset of atherosclerosis (35).

The increase in obesity and adipose tissue caused by diabetes leads to an increase in releasing of peptides such as leptin, adiponectin and vaspin from these tissues, while it is also observed the increase in production of some proinflammatory cytokines in the same tissues (18, 22, 41). It has been reported that interleukin-6 (IL-6) and TNF-α are released from adipose tissue and its plasma levels increase in parallel with adipose tissue increase (11, 25). In addition, it was claimed that there is a

correlation between these inflammatory cytokines and both endothelial dysfunction and the development of atherosclerosis (8, 49).

Melatonin, known as the hormone of darkness, has an important role in various functions on metabolic events as well as the regulation of the biological rhythm (31). It has been suggested that melatonin modulate lipoprotein metabolism and improve the negative changes in plasma triglyceride, total cholesterol, LDL and HDL levels in diabetic patients. On the other hand, it was suggested that melatonin has hypocholesterolemic effect by reducing cholesterol absorption from the intestines (12). It has been reported that melatonin administration to diabetic rats has positive effects on changes in plasma and erythrocyte GSH, triglyceride, cholesterol and HDL levels (28).

The aim of this study was to determine the effects of melatonin application on changes in plasma lipid profile caused by streptozotocin-induced diabetes.

MATERIAL AND METHOD

In this study, 32 adult male Wistar Albino rat was used. Rats were kept in proper conditions (heat, humidity and light) during the study period. The animals were divided into four groups. Standard rat diet was fed ad libitum to animals in all groups for 8 weeks. No application was made to animals in control group (K, n=6). The animals in the melatonin (M, n=6) group were intraperitoneally injected 50 mg/kg melatonin (Sigma-Aldrich, St. Louis, MO, USA) every day for 8 weeks. In diabetes group (D, n=9), diabetes was induced by subcutaneous injections of 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. Animals in Diabetes+Melatonin group (DM, n=10) were made diabetic by application of streptozotocin in the same way and then were intraperitoneally injected with 50 mg/kg melatonin during 8 weeks. Six hours after streptozotocin administration, rats were orally received 5% dextrose solution to prevent hypoglycemia for 3 days. One week after the streptozotocin injection, blood glucose levels were measured by glycometer (PlusMED Accuro, Taiwan) and rats which have 250 mg/dl or high blood glucose levels were included in the diabetic groups (group D and DM). One animal from the diabetes group died due to hypoglycemia during the study. At the end of 8 weeks, the TNF-α, Triglyceride, Total Cholesterol, LDL and HDL levels were determined in blood samples.

TNF-α level were determined with ELISA (Biotek ELx800, Biotek Instrumentations, Inc, Winooski, VT, USA) using sandwich enzyme-linked immunosorbent method via commercial kits (Elabscience), while Triglyceride, Total Cholesterol, LDL and HDL levels were determined the Abbott C8200 autoanalyzer using Abbott kits.

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 $p < 0.05$. The study protocol was approved by The Ethical Committee of Selcuk University Experimental Medicine Research and Application Center (Report no. 2017-15).

RESULTS

The effects of melatonin administration on plasma total cholesterol, triglyceride, some lipoprotein levels and TNF-α in streptozotocin-induced diabetic rats are summarized in Table 1.

TNF-α, a proinflammatory cytokine, was found to be significantly higher in diabetes group than the control group (Table 1, $p < 0.05$), whereas it was significantly lower in melatonin administrated diabetic rats than in the diabetes group (Table 1, $p < 0.05$). In the study, it was determined that plasma triglyceride level was significantly higher in rats with experimental diabetes than the control group (Table 1, $p < 0.05$). In diabetic rats, decrease in plasma HDL level (Table 1, $p < 0.05$) and increase in LDL level (Table 1, $p < 0.05$) were found to be significant compared to the control group, but the increase in total cholesterol level was not significant. With 50 mg/kg melatonin administration to diabetic rats, plasma HDL level were found to be significantly higher compared to diabetic animals (Table 1, $p < 0.05$) and LDL level were significantly lower than in diabetic animals (Table 1, $p < 0.05$). The changes in plasma total cholesterol and triglyceride levels with melatonin administration to diabetic rats were not important when compared to diabetes group.

Table 1. Effect of melatonin on total cholesterol, triglyceride, LDL, HDL and TNF-α levels in streptozotocin-induced diabetic rats (Mean±SE).

	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TNF-α (pg/ml)
K	87.33±6.63[ab]	62.50±5.13[b]	43.83±1.87[bc]	39.67±3.65[a]	91.54±3.96[c]
M	80.83±6.29[b]	61.17±7.31[b]	39.50±1.18[c]	44.50±4.96[a]	89.28±7.14[c]
D	105.50±4.92[a]	86.50±5.16[a]	64.90±3.93[a]	28.10±3.43[b]	127.49±5.35[a]
DM	92.11±6.27[ab]	79.56±7.09[ab]	51.78±4.70[b]	41.78±3.46[a]	109.42±4.56[b]

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

DISCUSSION

Diabetes affecting millions of people worldwide is characterized by high blood glucose levels, insulin resistance or insufficiency. Some of the complications responsible for the mortality are considered as triopaty (retinopathy, neuropathy and nephropathy), dyslipidemia and cardiovascular diseases (5, 39). In recent years, there has been a growing interest in the use of various alternative products for the treatment and prevention of diabetes (3, 4, 20, 39, 42). Melatonin depend on its many physiological effects has been used as an option in preventions of diabetes and its complications (28, 43).

TNF-α is a proinflammatory cytokine which is produced by macrophages and adipose tissue. The plasma level of TNF-α is reported to be high in diabetes studies conducted in human and rat (6, 10, 13, 15, 32, 40, 46). It is stated that TNF-α production and release increased in response to systemic inflammation plays a role in dyslipidemia and vascular pathophysiology related to diabetes (44, 45). It is reported that increased TNF-α in diabetes further exacerbates the current situation by inhibiting the kinase activities that form the insulin signaling pathway in skeletal muscles, fat tissue, endothelial cells and other tissues (1, 37). It has been shown that TNF-α increase the levels of triglyceride and very low density lipoprotein (VLDL) as it affects lipolysis in mouse, rat and human fat cells (13, 36, 49). One of the best known properties of melatonin, also known as the hormone of darkness, is reported as its effect on anti-inflammatory mechanisms (43). It has been suggested that melatonin inhibits proinflammatory cytokines in polymorphic nuclear leukocytes, endothelial cells and colitis, thereby alleviating tissue damage and cell migration due to inflammation (17, 30, 43). The effects of melatonin on proinflammatory cytokines, especially TNF-α, are attributed to the inhibition of mRNA expression of these cytokines (21). In paralelly, administration of oral melatonin has been reported to modulate overexpression of TNF-α (9, 38). The decrease in plasma TNF-α level with melatonin administration to diabetic rats can be explained with the mechanisms stated above. The TNF-α level in diabetic animals treated with melatonin was still higher than both control and melatonin group levels. This can be accepted a sign of systemic inflammation relatively continouied due to diabetes.

Plasma triglyceride levels were significantly higher in rats with experimentally induced diabetes than the control group (Table 1, p<0.05). In diabetic rats, decrease in plasma HDL level (Table 1, p<0.05) and increase in LDL level (Table 1, p<0.05) were found to be significant compared to the control group. With 50 mg/kg melatonin administration to diabetic rats, plasma HDL level were found to be significantly higher compared to diabetic animals (Table 1, p<0.05) and LDL level were significantly lower than in diabetic animals (Table 1, p<0.05). Melatonin administration to rats with diabetes resulted in obviously improving HDL and LDL levels but the effects of melatonin on plasma triglyceride and total cholesterol levels were found to be limited. There are some reports that hyperglycemia generally caused hypertriglyceridemia and hypercholesterolemia (48). The findings obtained from our study appear to be consistent with the data of researches conducted on the same subject (19, 24, 48). The positive changes observed in the plasma lipid parameters by the administration of melatonin are attributed to the reasons such as suppressed tissue lubrication (34, 47), increased lipoprotein lipase activity and increased insulin sensitivity resulting in decreased

of lipolysis in adipose tissue (34). Mechanisms such as inhibition of cholesterol absorption (12) and synthesis (2), increased conversion of cholesterol to bile acids, increased LDL receptor activity (2, 29), and inhibition of metabotropic receptors involved in the transport of fatty acids (7) are considered among the corrective effects of melatonin on the lipid profile (27).

CONCLUSION

When considering significant changes obtained in TNF-α, HDL and LDL levels with melatonin application and partial positive changes in total cholesterol and triglyceride levels, it was thought that melatonin application might have a corrective effect on dyslipidemia seen in diabetes.

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