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Antioxidant Effects of Pentoxifylline and Melatonin in the Alloxane-Induced Diabetic Mice

[Alloksanla Diyabet Oluşturulan Farelerde Pentoksifilin ve Melatoninin Antioksidan Etkileri]

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ÖZET

Bu çalışmada, alloksanla diyabet oluşturulan farelerin karaciğer ve böbrek dokularında pentoksifilin (PTX) ve melatoninin (MLT) antioksidan etkilerinin araştırılması amaçlandı. Her grupta 10 fare olmak üzere 4 grup oluşturuldu ve çalışma 8 hafta boyunca sürdürüldü; diyabetik olmayan kontrol grubu, tedavi verilmeyen diyabetik grup, PTX'le tedavi edilen (50 mg/kg/gün sc) diyabetik grup, MLT'le tedavi edilen (10 mg/kg/gün sc) diyabetik grup. PTX vücut ağırlığında artma ve kan glukoz düzeyinde azalmaya yol açarken ($p<0.01$), MLT sadece vücut ağırlığında artışa neden oldu ($p<0.01$). Lipid peroksidasyon son ürünü olan malondialdehit (MDA) seviyesi karaciğer dokusunda, diyabetik farelerde, diyabetik olmayan farelere göre anlamlı artış gösterirken ($p<0.01$), PTX ve MLT'le tedavi edilen farelerde ise tedavi verilmeyen diyabetik farelere göre anlamlı azalma gösterdi ($p<0.01$). PTX ve MLT'le tedavi edilen farelerde böbrek MDA seviyesi, tedavi verilmeyen diyabetik farelere göre azalma göstermesine rağmen bu farklılık istatistiksel açıdan anlamlı değildi ($p>0.05$). PTX'le tedavi edilen farelerde böbrek ve karaciğer glutatyon peroksidaz (GSH-Px) aktivitesi diyabetik olmayan farelere göre anlamlı artış gösterirken ($p<0.05$), böbrek GSH-Px aktivitesi PTX'le tedavi edilen farelerde, tedavi edilmeyen diyabetik farelere göre anlamlı artış gösterdi ($p<0.05$). Bununla birlikte, tedavi verilmeyen diyabetik farelerle karşılaştırıldığında, PTX ve MLT ile tedavi edilen farelerin karaciğer GSH-Px, karaciğer ve böbrek dokusu katalaz (CAT) ve superoksit dismutaz (SOD) aktiviteleri arasında anlamlı bir farklılık yoktu ($p>0.05$). Diyabetik olmayan ve diyabetik fareler arasında böbrek MDA düzeyi, GSH-Px ve CAT aktivitesi ve yine karaciğer ve böbrek SOD aktivitesi arasında da anlamlı bir farklılık yoktu ($p>0.05$). Bu sonuçlar, diyabetin karaciğer ve böbrek dokularında oksidatif streste artışa neden olabileceğini, pentoksifilin ve melatonin uygulamasının serbest radikal önleyici etkisiyle bu dokularda koruyucu etki gösterebileceğini düşündürmektedir.

Anahtar Kelimeler: Diyabetes mellitus, oksidatif stres, melatonin, pentoksifilin.

ABSTRACT

It is aimed to investigate antioxidant properties of pentoxifylline (PTX) and melatonin (MLT) on the liver and kidney tissues in alloxane-induced diabetic mice. Animals were divided into four groups of 10 animals each as follows: Non-diabetic mice, non-treated diabetic mice, diabetic mice treated with PTX (50 mg/kg/day sc), and diabetic mice treated with MLT (10/mg/kg/day sc), for 8 weeks. PTX caused an increase in body weight and a decrease in blood glucose level ($p<0.01$), while MLT caused an increase only in body weight as compared to the initial values ($p<0.01$). In non-treated diabetic mice the liver malondialdehyde (MDA) levels were increased significantly compared with non-diabetic mice ($p<0.01$), while the liver MDA levels in mice treated with PTX and MLT were decreased significantly compared with non-treated diabetic mice ($p<0.01$). Although the kidney MDA levels in mice treated with PTX and MLT were decreased when compared with non-treated diabetic mice, this difference was not found to be statistically significant ($p>0.05$). While the kidney and liver glutathione peroxidase (GSH-Px) activity in mice treated with PTX increased significantly when compared to non-diabetic mice ($p<0.05$), the kidney GSH-Px activity in mice treated with PTX increased significantly when compared to non-treated diabetic mice ($p<0.05$). However, the liver GSH-Px, kidney and liver catalase (CAT) and superoxide dismutase (SOD) activities in mice treated with PTX and MLT were not differed from those 93in non-treated diabetic mice ($p>0.05$). There were no also significant differences between non-treated diabetic mice and non-diabetic mice both in MDA levels, GSH-Px and CAT activities of kidney, and also SOD activities of the kidney and liver ($p>0.05$). The results suggest that diabetes may increase oxidative stress in liver and kidney tissues and pentoxifylline and melatonin might have a protective affect via them free radical-scavenging properties in these tissues.

Key Words: Diabetes mellitus, oxidative stress, melatonin, and pentoxifylline.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and insufficiency of secretion or action of endogenous insulin. While exogenous insulin and other medications can control many aspects of diabetes, numerous complications affecting the vascular system, kidney, retina, lens, peripheral nerves, and skin are common and extremely costly in terms of longevity and quality of life (1).

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes mellitus and its complications (2). Diabetes is usually accompanied by increased production of free radicals (3) or impaired antioxidant defences (4). Under normal conditions, the body's natural defence enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), make free radicals innocuous by reducing superoxide radicals and peroxides, concurrently oxidizing glutathione (5). While hyperglycaemia engenders free radicals, on the other hand it also impairs the endogenous antioxidant defence system in many ways during diabetes (6). Antioxidant defence mechanisms involve both enzymatic and non-enzymatic strategies.

Pentoxifylline (PTX) [3,7-dimethyl-1-(5-oxohexyl) xanthine] is a methyl–xanthine derivative that has been used for its regulator effects on the blood flow for the treatment of peripheral vascular and , cerebrovascular diseases, and a number of other conditions involving a defective regional microcirculation (7). PTX can enhance the chemotactic response of neutrophils, but may inhibit phagocytosis and superoxide production by neutrophils and monocytes (8, 9).

Melatonin (MLT), [N-acetyl-5-methoxytryptamine], is an indole amine synthesized during the night in the pineal gland (10). Its biological antioxidant activity is well known and it stands out as a powerful neutralizer of hydroxyl free radical (11). This is because of: (a) its easy diffusion through membranes; and (b) it does not need specific receptors to carry out its antioxidant activity. In addition, MLT regulates the activity and gene expression of antioxidant and pro-oxidant enzymes (12, 13).

In this experimental study, the effects of PTX and MLT treatment on antioxidant balance in alloxane induced diabetic mice were investigated.

MATERIALS AND METHODS

Twenty female and twenty male mice weighing 17.6-47.6 gram (g) were used in this study. Mice were kept under standardised conditions of food, water, light and temperature. The approval of Yuzuncu Yil University, School of Medicine Animal Ethics Committee was obtained. Diabetes was induced by intraperitoneal (i.p) injection of alloxane (ALX) (200 mg/kg/day for three days) freshly prepared in saline (50 mg/ml) in animals fasted overnight. Control mice received the same volume of saline. Blood samples for measurement of glucose were obtained from the tail vein. The mice injected with ALX were considered as diabetics if the fasting blood glucose levels were >200 mg/dl. PTX and MLT treatment was started seven days after the last alloxane injection. The mice were divided into four groups each containing 10 animals (five female and five male mice):

Non-diabetic mice; Age and sex-matched control mice,

Non-treated diabetic mice; ALX-induced untreated diabetic mice,

Diabetic mice treated with PTX; Diabetic mice treated with 50 mg/kg/day of PTX (Trental, Aventis Pharma, İstanbul) injected subcutaneously (sc) for 8 weeks and,

Diabetic mice treated with MLT; Diabetic mice treated with 10 mg/kg/day of MLT injected sc for 8 weeks period. All mice were maintained on standard mice chow and tap water ad lib.

TISSUE SAMPLE PREPARATIONS

At the end of the 8 weeks period, animals were sacrificed after an overnight fasting, by exsanguinations under ether anaesthesia. The liver and kidney tissues of each animal were removed, cleaned, dried and processed for biochemical measurements. The homogenates were prepared on ice in the ratio of 1:4 (tissue weights: buffer volume (ml)), centrifuged at 10,000 X g for 20 min at 4 °C and kept at -70 °C for one month. Tissues were homogenized in 0.1 M phosphate buffer with 0.1 mM EDTA, pH 7.0.

BLOOD AND TISSUE ANALYSIS

Blood glucose concentrations were measured by Optium MediSence glucometer (Abbott Lab, USA).

The levels of malondialdehyde (MDA), as an end product of lipid peroxidation, were measured fluorometrically in tissue homogenates (14).

Cu, Zn-SOD activities of liver and kidney tissues were determined by the method of Sun et al. (15) based on the inhibition of nitroblue tetrazolium using the xanthine-xanthine oxidase system as a superoxide generator. The absorbance of the reduction product (formazone) was measured at 560 nm. Superoxide dismutase activity was measured as the degree of inhibition of this reaction.

CAT activities of liver and kidney tissues were determined by Goth's colorimetric method (16), in which homogenate was incubated with H_2O_2 substrate. The enzyme reaction was stopped by the addition of ammonium molybdate. The intensity of the yellow complex formed by molybdate and H_2O_2 was measured at 405

Table I. The glucose levels and body weights in the alloxane-induced diabetic mice (mean±SD).

Parameters	Non-diabetic mice	Non-treated diabetic mice	Diabetic mice treated with PTX	Diabetic mice treated with MLT
Blood glucose (0. days)	78.8±12.8	362.2±195.2	262.36±76.85	252.06±52.47
Blood glucose (60. days)	81.12±12.36	329.0±144.73	178.94±125.15[a**]	247.53±131.82
Initial body weight (0. days)	33.8±4.23	27.48±5.44 [b**]	24.95±6.40 [b**]	26.96±4.71 [b**]
Final body weight (60. days)	33.1±4.15	27.28±10.07	34.94±6.95 [a **, c*]	37.49±6.0 [a **, c*]

Glucose (mg/dl), body weight (g)

*p<0.05, **p<0.01, [a]compared to initial levels; [b]compared to non-diabetic mice; [c]compared to non-treated diabetic mice.

Table II. Effect of PTX and MLT on the liver and kidney tissue contents of MDA, GSH-Px, SOD and CAT in experimental mice (mean±SD).

Parameters	Non-diabetic mice	Non-treated diabetic mice	Diabetic mice treated with PTX	Diabetic mice treated with MLT
Liver MDA (μmol/g prot)	74.2 ± 13.05	136.18±46.74[a**]	67.54±40.13[b**]	68.72±13.27[b**]
Liver GSH-Px (U/g prot)	65.16±9.15	128.91±61.1 [a*]	118.46±43.05 [a*]	108.33±40.11
Liver SOD (U/mg prot)	10.51±7.06	28.38±38.66	20.5±18.58	31.46±16.0
Liver CAT (kU/g prot)	8.23± 2.28	18.01±9.78[a*]	14.65±4.94	16.03±4.82[a*]
Kidney MDA (μmol/g prot)	77.26± 13.18	133.21±52.2	95.36±73.46	82.93±32.74
Kidney GSH-Px (U/g prot)	233.49±91.03	236.01±127.53	354.3±119.92 [a*,b*]	257.38±57.97
Kidney SOD (U/mg prot)	6.95±6.73	55.23±49.56	73.79±45.44[a*]	76.78±28.43 [a*]
Kidney CAT (kU/g prot)	40.54± 20.11	33.34±21.66	47.21±19.61	41.2±8.15

*p<0.05, **p<0.01, [a]compared to non-diabetic mice; [b]compared to non-treated diabetic mice.

nm.

GSH-Px activities of liver and kidney tissues were determined by the modified method of Paglia and Valentine (17). In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted into the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

Protein content of homogenates was measured by the method of Lowry (18).

STATISTICAL ANALYSIS

The data are expressed as mean ± SD. The comparisons of initial and final glucose and body weight values were analysed with paired t test. Kolmogrov-Smirnov Goodness of Fit Test was used to control whether the distribution of parameters was normal or not. Then the groups of data were compared using the analysis of variance (One-way ANOVA) followed by Tukey's multiple comparison tests.

RESULTS

The blood glucose levels and body weights of mice included in the study are presented in Table I. The body weights of non-treated and treated diabetic mice were significantly lower than those of non-diabetic mice (p<0.01) before the treatment with PTX and MLT,. Nevertheless the body weights of diabetic mice after treatment with PTX and MLT were significantly higher than those of non-treated diabetic mice (p<0.05). As compared to their initial values, the body weights of diabetic mice treated with PTX and MLT were significantly increased at the end of the treatment period (p<0.01). Also, as compared to their initial values, the blood glucose levels of diabetic mice treated with PTX were significantly decreased at the end of the treatment period (p<0.01).

Table II shows the effect of PTX and MLT treatment on the antioxidant systems in liver and kidney tissues. In non-treated diabetic mice the liver MDA levels increased significantly when compared to non-diabetic mice (p<0.01), while the liver MDA levels in diabetic mice treated with PTX and MLT decreased significantly when compared to non-treated diabetic mice (p<0.01). In diabetic mice treated with PTX, the kidney and liver GSH-Px activities were found to be increased than those of the non-diabetic mice (p<0.05, both), while the kidney GSH-Px activity in mice treated with PTX also increased significantly when compared to non-treated diabetic mice (p<0.05). The liver CAT activity in non-treated diabetic mice and in mice treated with MLT, and

also the kidney SOD activity in mice treated with PTX and MLT were significantly increased compared to non-diabetic mice ($p<0.05$, all). The liver GSH-Px, kidney and liver CAT and SOD activities in mice treated with PTX and MLT were not different from those in non-treated diabetic mice ($p>0.05$). No significant difference was found between the non-treated diabetic and non-diabetic mice corresponding to MDA levels, GSH-Px and CAT activities of kidney, and also SOD activities of the kidney and liver ($p>0.05$).

DISCUSSION

Diabetes can be produced in animals by drugs or chemicals such as alloxan and streptozotocin. The mechanisms of the effects of these two drugs are different, but they both result in the production of active oxygen species which have cytotoxic effect on pancreatic beta cells that are responsible for insulin production. Oxygen free radicals are not only involved in the cause of diabetes, but also appear to play a role in some of the complications seen in the long-term treatment of diabetes (10, 19).

The present study showed that PTX treatment caused a decrease in blood glucose levels when compared to the initial values. It was previously demonstrated that millimolar concentrations of PTX stimulate glucose-induced insulin secretion in perfused rat pancreas preparations (20). PTX was reported to exert its insulin stimulatory effects by inhibiting cyclic adenosine monophosphate (cAMP), a known modulator of insulin secretion (21). It may also enhance glucose-induced insulin secretion through its anti-inflammatory actions either by inhibition of macrophage activation and release of proinflammatory cytokines or by preventing cytokine-induced loss of β-cell functions. In addition, the effects of PTX and MLT on the enhanced body weight suggest that the weight loss associated with diabetes mellitus might be neutralized by PTX and MLT. This effect may be associated with various mechanisms such as inhibition of TNF production by PTX, and decreased plasma leptin levels by MLT. Circulating leptin levels were reported to be decreased by MLT (22). However, the data of the present study is not definitive since these factors were not investigated in this study.

In the present study, MDA levels of the liver in diabetic animals treated with PTX and MLT were found to be lower than those in non-treated diabetic group. Although the MDA level of the kidney in diabetic animals treated with PTX and MLT were lower than those in non-treated diabetic group, this decrease was not found to statistically significant. These results provided evidence for the free radical-scavenging properties of these agents. PTX was shown to improves erythrocyte deformability, to decreases platelets and neutrophils plugging, and to reduces the blood viscosity. It was also reported that it improves the tissue oxygenation due to improved microcirculatory blood flow and decreased neutrophil adhesiveness. PTX is known to possess anti-inflammatory properties that are probably related to their ability to suppress oxygen radical production or scavenge reactive oxygen species. PTX is known to increase intracellular cAMP, which in turn inhibits phospholipase A_2 and phospholipase C activity, so reducing substrate availability for peptide-leukotriene production (9, 23-25). Some studies have shown that MLT has antioxidant properties and prevents lipid peroxidation (26-28). MLT was found to provide indirect production as well as the direct one against free radical attack since it stimulates antioxidative enzymes. As reviewed by Reiter et al. (12) MLT was suggested to protects tissues against oxidative injuries in vivo as well as in vitro. The protective effect of MLT as shown in the current study is similar to that reported in previous studies (10, 29-30).

Liver and kidney are essential tissues where important complications of diabetes mellitus occur. It was shown that the severity of diabetic complications in tissues is related to the damage in their oxidative-antioxidative systems (31). There is no study evaluating the cooperative effects of PTX and MLT on the antioxidative status of tissues that are expected to be affected by diabetes. The concerted actions of various antioxidant enzymes that keep the concentration of free radicals relatively low are overwhelmed in states of oxidative stress, such as diabetes. In the present study, it was found that PTX and MLT had some effects on antioxidant enzyme activities in liver and kidney tissues. PTX therapy caused a significant increase in kidney GSH-PX level in treated diabetic animals when compared with that in non-treated diabetic animals. Glutathione ia known to be involved in GSH-Px pathway and to protects tissues from free radical damage. whereas GSH-Px is known to be capable to decrase hydrogen peroxide and organic hydroperoxides (9). The increase of GSH-Px activity may suggests a compensatory response to oxidative stress due to an increase in endogenous hydrogen peroxide production. However, SOD and CAT levels of tissues of diabetic mice treated with PTX and MLT were not found to be different from those in non-treatment diabetic group. This may be originated from different metabolic actions of these tissues and their different responses to oxidative stress.

In conclusion, it is speculated that PTX and MLT administration would be beneficial in the treatment of diabetes as an antioxidant and a free radical scavenger in controlling glucose levels and body weights. However, further studies are needed to explain the importance of pentoxifylline and melatonin in the therapy of diabetes mellitus and to show its possible effects to prevent the complications of diabetes mellitus on the liver and kidney.

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