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Melatonin supplementation plus exercise behavior ameliorate insulin resistance, hypertension and fatigue in a rat model of type 2 diabetes mellitus



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ABSTRACT

Introduction: The objective was to investigate the effects of melatonin and exercise on insulin resistance (IR), hypertension and fatigue syndrome in a rat model of type 2 diabetes mellitus (T2DM).

Materials and methods: Rats were divided into 5 groups namely normal control (NC), T2DM control group (DC), diabetes plus exercise (DE), diabetes plus oral melatonin supplement (DM) and diabetes plus melatonin and exercise (DME) groups. Melatonin was administered orally 5 mg/kg twice daily and 40 min swimming/day 5 days/week were regimented after diabetes induction.

Results: Blood pressure, fasting blood glucose, insulin, IR, serum leptin, lipid profiles, inflammatory cytokines, lipid peroxidation increased significantly ($P < 0.01$) while serum adiponectin, antioxidant activities (superoxide dismutase, glutathione), exercise performance significantly decreased ($P < 0.001$) in the DC group compared with the control group. Combined effects of exercise and melatonin ameliorated markedly hypertension, IR, biochemical alteration induced by diabetes and significantly increased exercise performance ($P < 0.01$). The expression glucose transporter type 4 (GLUT4) mitochondrial biogenesis related proteins such as peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1 α), nuclear respiratory factor (NRFs) and mitochondrial transcription factor-A were up-regulated skeletal and cardiac muscle in the DME group.

Conclusion: Melatonin supplementation in combination with exercise behavior may ameliorate IR, hypertension and exercise performance or fatigue possibly by improving antioxidative activities, hyperlipidemia, inflammatory cytokines via up-regulation of GLUT4, PGC-1 α and mitochondrial biogenesis in T2DM rats.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder manifested primly by persistent hyperglycemia and insulin resistance (IR) due to abnormal production of insulin or lack of cellular up taking [1]. T2DM is rapidly increasing and reaching epidemic proportions worldwide due to modern socioeconomic life style, such as physical inactivity, watching television for a long time without exercise, higher dietary glycemic load and trans-fat intake, smoking and alcohol consumption etc. [2,3]. Hyperglycemia, IR, hyperlipidemia, hyper leptinemia, increased oxidative

stress, inflammatory cytokines, hypertension and fatigue are clinical signs and symptoms of human type 2 diabetes mellitus [4]. Cardiovascular complications are one of the leading causes of death in diabetic patients [5]. Fatigue had been reported in up to 60% of diabetic patients which are more distinctly correlated with DM. Fatigue refer to abnormal exhaustion, lack of stamina to complete a task or decrease ability in physical activity [6]. This symptom is not merely indicates clinical sign but also the severity of disease status and complications in diabetes mellitus patient [7].

Melatonin is primarily synthesized from the pineal gland and it metabolites have powerful antioxidant properties and potent scavenging activities of toxic free radicals [8]. It manipulates wide variety of biological processes including circadian rhythms, neuroendocrine, cardiovascular and immune functions [9]. It has anti-hypertensive (9), hypolipidemic and anti-obesity effects [10,11]. Exercise is frequently recommended as a useful way to

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lower blood glucose for the management of diabetes and obesity [12,13]. There is a very wide number of studies reported dealing with melatonin and diabetes [14]. However, a few studies [1,12] that have examined the actions of melatonin in combination with exercise as they relate to type 2 diabetes but still many things remain unknown. Due to long term existing nature of diabetes, oral route for melatonin administration was selected to avoid discomfort of patient. The aim of this study was to investigate the ameliorative effect of oral melatonin supplementation plus exercise on T2DM rat model and on its complications such as, IR, hypertension and decreased exercise performance or fatigue syndrome.

2. Materials and methods

2.1. Animals and experimental design

Male white Sprague-Dawley rats (Orient Bio, Gapyeong, Gyeonggi-do, Korea) were used for this study. The rats were housed in controlled environment with temperature of $(23 \pm 2)^\circ\text{C}$ and humidity of $(50 \pm 5)\%$ with a 12–12 h light-dark cycle. Food and water were available ad libitum before started experiment. After a week of adaptive feeding, average body weight was 222 ± 3 g. The design of type 2 diabetic mellitus rat model such as dosage and timing was adapted and modified as described previously [15]. To induce diabetes the rats were fed with a high-fat diet for 45 days, while the normal control groups were given regular diet. Type 2 diabetes mellitus (T2DM) with 60% high fat diet for 45 days and STZ (40 mg/kg body weight in 0.05 M citrate buffer; pH 4.5; Sigma; after 15 min Nicotinamide 200 mg/kg body weight). The 75 rats were equally divided in to five groups namely, NC group (normal control): was treated with saline in a matched volume. DC group (Type 2 Diabetes mellitus control); DE group (Diabetic rats regimented exercise); DM group (Diabetic rats supplemented only with melatonin without exercise) and DME (Diabetic rats supplemented with melatonin and regimented with exercise). The animals were placed individually in cages. Melatonin (Sigma Chemical Co, St Louis, MO, USA) dissolved in 0.1% ethanol solution and administered at the dose of 5 mg/kg body weight twice daily by oral gavage. Melatonin solution was prepared freshly every day. Exercise was regimented 40 min/day and 5 days/week. All experimental protocols employed herein were approved by the committee on the care of laboratory animal resources, KNOTUS Co., Ltd, Korea, (Certificate number: IACUC 16-KE-100) and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication no. 85-23, revised 1996).

2.2. Swimming performance and fatigue test

Exercise regimentation was done by the modified technique of Rahman et al. [16]. The swimming pool consists of a glass chamber (80 cm in height, 100 cm in length, and 80 cm in width) filled with $36 \pm 1^\circ\text{C}$ warm water 60 cm high, with a heating system and an air pumping system. To prevent floating during swimming, water bubbles were sparingly produced by tubes connected to the air pump system. The temperature of the water within the glass chamber was kept at $36 \pm 1^\circ\text{C}$ via a thermostatically controlled heater located at the base of the chamber. The 3 rats together were forced to swim 40 min. After 5 weeks of regular exercise sessions, five rats from each group were selected for the swimming performance test. The NC, DC and DM groups had been trained every second day, 10 min per session, in the last 2 weeks before completion. Three fatigue tests were performed every other day as previously described [17].

2.3. Measurement of fasting blood glucose level

The fasting blood glucose (FBG) levels were determined with a blood glucose meter (ACCU-CHEK[®] Active, Roche Diagnostics, Mannheim, Germany). These measurements were performed every 15 days.

2.4. Blood pressure measurement

Blood pressure was measured using the noninvasive tail cuff plethysmography method (IITC Inc., Life Science Instruments, Woodland Hills, CA, USA) 15 days interval. Blood pressure was measured under resting, conscious condition in a climate-controlled room (23°C). Rats were trained by measuring systolic blood pressure (SBP) daily for 5 days to avoid stress-induced SBP fluctuations. During this acclimation period, the cuff was inflated and deflated several times to adjust the animals to the procedure. The SBP values were averaged from five consecutive cycles per day obtained from each rat.

At the end of experiment animals were anaesthetized by Zoletil 50 (Teletamine HCl, 125 mg and Zolezepum 125 mg) (Virbac Laboratories, Carros, France) and 2% Rompun (Xylazine HCl) (Bayer, Leverkusen, Germany) combination (3:1) by injecting intraperitoneally 1 ml/kg body weight. The right carotid artery of each rat was cannulated for the measurement of heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MBP). A cannula outer dimension of 0.96 mm and inner dimension of 0.58 mm (fisher Scientific) was filled with heparinized saline and inserted in carotid artery and connected to Biopack computerized transducer system (Biopack, Goleta, CA) and then arterial pressure was recorded by using the acknowledge 3.5 software (East Palo Alto, CA, USA).

2.5. Measurement of serum biochemical parameters

Blood was collected from the caudal vena cava immediately following sacrifice, serum was separated by centrifugation at 3000 r/min for 10 min and stored at -20°C until analysis. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low density lipoprotein (LDL), free fatty acid (FFA) levels were determined with a Hitachi 7180 instrument (Hitachi, Tokyo, Japan). The serum level of very low density lipoprotein cholesterol (VLDL) was calculated using the Friedewald formula: $\text{VLDL} = \text{TG}/5$ [18]. The atherogenic index (AI), a useful determinant of cardiovascular is used by TC/HDL ratio [19]. Plasma leptin and adiponectin concentrations were measured using a rat leptin ELISA kit (Assay Designs, Inc., USA) and Rat Adiponectin ELISA kit (ALPCO Diagnostics, Windham, NH, USA) respectively. Fasting serum insulin levels were measured with a rat insulin enzyme linked immune absorbent assay kit (ALPCO Diagnostics, Windham, NH, USA) according to the manufacturer's protocol. Insulin resistance = $[\text{glucose (mmol/l)} * \text{Insulin (IU/L)}] / 22.5$.

The serum tumor necrotic factor- α (TNF- α) protein levels were measured with TNF- α (Rat) enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostics, USA) and interleukin-6 (IL-6) were measured with Rat IL-6 ELISA (ALPCO Diagnostics, USA) according to the manufactures protocol. TNF- α and IL-6 measurements were standardized with sample proteins and the plasma volume.

Concentrations of malondialdehyde (MDA) in serum and cardiac tissue were measured with an OXI-TEK TBARS kit (Enzo Life Sciences Incorporated). Reaction products were quantified by measuring the absorbance at 532 nm according to the manufacturer's suggested protocol. Serum and tissue levels of superoxide dismutase (SOD) were quantified using a SOD activity kit (Sigma Chemical Co, St Louis, MO, USA) by measuring the absorbance of

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