

ORIGINAL

Role of Ketotifen on metabolic profiles, inflammation and oxidative stress in diabetic rats

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Abstract. We aim to explore effects of Ketotifen on metabolic profiles, inflammation and oxidative stress. Sprague Dawley (SD) male rats were randomly divided into normal control group (NC) and experimental groups, and experimental group rats were fed with high-sugar and fat diet for 6 weeks. Then, experimental group rats were divided into diabetes group (DM) and ketotifen treatment group (KT). KT group was given ketotifen *via* Intragastric for 8 weeks with the dosage of 0.09 mg/kg/d. Fasting plasma glucose (FPG) was measured using glucose oxidase-phenol amino phenazone method. Fasting insulin (FINS), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were tested by enzyme-linked immunosorbent assay. Malondialdehyde (MDA) and superoxide dismutase (SOD) were quantified by spectrophotometer method. Before Ketotifen administration, compared with NC group, DM and KT groups showed significantly high levels of body weight, FPG, FINS, HOMA-IR, TC, TG, LDL, IL-6, TNF- α and MDA, and lower levels of HDL and SOD (All $p < 0.05$). After 4 weeks of Ketotifen administration, levels of body weight, FPG, FINS, HOMA-IR, TC, TG, LDL, IL-6, TNF- α in KT group decreased significantly, and levels of HDL and SOD elevated significantly (All $p < 0.05$). After 8 weeks of Ketotifen administration, levels of body weight, FPG, FINS, HOMA-IR, TC, TG, LDL, IL-6, TNF- α and MDA in KT group decreased more obviously, and levels of HDL and SOD elevated significantly further (All $p < 0.05$). Ketotifen improved metabolic profiles, and ameliorated status of inflammation and oxidative stress.

Key words: Ketotifen, Diabetes mellitus, Mast cell, Inflammation, Dyslipidemia

TYPE 2 DIABETES, one type of metabolic disorders, loads a great burden on individuals and society.

Submitted Oct. 10, 2016; Accepted Dec. 29, 2016 as EJ16-0458
Released online in J-STAGE as advance publication Mar. 17, 2017
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Hyperglycemia itself and its complications including dyslipidemia, hypertension and atherosclerosis, increase morbidity and mortality for individuals [1, 2]. Many pathophysiologic mechanisms underlie type 2 diabetes. Mounting evidence revealed that chronic inflammation and oxidative stress have been associated with the development of type 2 diabetes [3-7]. In recent years, progress in anti-inflammatory and oxidative stress therapy for diabetes is remarkable [8, 9], emerging a large stream of new therapeutic agents.

Ketotifen, one type of classical mast cell stabilizer, is widely and commonly used for treating allergic

diseases. Interestingly, it has been applied to non-allergic diseases, like improving sperm quality [10], irritable bowel syndrome [11] and reducing joint capsule fibrosis [12]. In addition, it has been suggested ketotifen has a novel medication for diabetes through stabilization of mast cells in animal model and human [13, 14]. However, its mechanisms remain largely unknown. Therefore, more researches are needed for this phenomenon and its possible mechanisms.

In present study, we aimed to explore the role of ketotifen on the metabolic profiles, inflammation and oxidative stress.

Materials and Methods

Drugs, reagents and equipment

Ketotifen was purchased from Zhejiang Nanyang Pharmaceutical Co (lot number 120306). STZ (purity > 98%) was purchased from Sigma. High-sugar and high-fat diet contained 20% lard, 5% sucrose, 1% cholesterol and 74% basal diet. IL-6 and TNF- α kit was purchased from Beijing biological Engineering Co, Ltd. SOD kit and MDA kit purchased from Nanjing Jiancheng Bioengineering Institute; Insulin, TG, TC, HDL-C, LDL-C assay kit was purchased from Zhejiang Dongou biological Engineering Co., Ltd; The blood glucose meter was purchased from Johnson company.

Animal model and specimen collection

The study was approved by the Shanghai Laboratory Animal Ethics Committee Institute of Materia Medica. SD male rats (6 to 8 weeks old, weighing 160 to 180g) were provided by the Experimental Animal Lake Hayes limited liability company, and were housed in Wenzhou Medical College Experimental Animal Center, SPF. Adaptive feeding lasted for one week. All the SD male rats were then randomly divided into normal control group (NC) (n=16) and the experimental group (n=48). Starting from the second week, the experimental group rats were fed high-sugar and high-fat diet lasting for six weeks. Fasting was given for the rats in the experimental group for 12h but with unlimited water. And then intraperitoneal injection of fresh citrate buffer (pH 4.5) configuration of STZ 35 mg/kg was made for the rats in the experimental group. Three to seven days later, fasting plasma glucose was tested for these rats, whose fasting plasma glucose level was high than > 13 mmol/L, indicating the success of diabetic model. The success rate was about 70%, and then dividing these

randomly into the diabetic group (DM) (n=16) and the ketotifen treatment group (KT) (n=16). The experimental group rats were given continually high-sugar and high-fat diet. The dosage of ketotifen was calculated based on the "common animal and human area ratio". The dosage of ketotifen dosage was 0.09 mg/kg/d and paired with saline 0.04 g/L. The KT group rats were given ketotifen through Intragastric administration. The normal control group and diabetic control group were given in the same way with saline instead for 8 weeks. These rats were killed at three time points, including at the 0, 4 and 8 weeks. FPG and FINS were tested before killed. TC, TG, HDL-C, LDL-C, IL-6, TNF- α and MDA levels and SOD activity were determined after decapitation.

Biochemical measurements

Plasma glucose was measured using glucose oxidase-phenol amino phenazone method. IL-6 and TNF- α were tested by an enzyme-linked immunosorbent assay (ELISA). MDA and SOD concentrations were quantified by using spectrophotometer method. FINS, TC, TG, HDL-C and LDL-C were also evaluated by using ELISA.

Statistical analysis

All statistical analyses were performed by using SPSS17.0 software package (IBM, USA). The comparisons among different groups were analyzed by using analysis of variance (ANOVA) test and further between-group comparison was done using the LSD test. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (mIU/mL) \times fasting plasma glucose (mmol/L) / 22.5. The statistical tests were two-sided, a $p < 0.05$ was considered statistically significant.

Results

Effect of ketotifen on body weight and glucose metabolism

Compared with normal control group, both DM and KT groups showed significantly high levels of body weight, fasting plasma glucose, fasting insulin and HOMA-IR before ketotifen treatment (All $p < 0.05$). After four weeks of ketotifen treatment, body weight, fasting plasma glucose, fasting insulin and HOMA-IR in KT group decreased significantly when compared with DM group (All $p < 0.05$). Also, body weight,

fasting plasma glucose, fasting insulin and HOMA-IR levels of KT group decreased further after eight weeks of ketotifen treatment ($p < 0.05$) (Fig. 1, Supplementary Fig. 1 and Fig. 2).

Effect of ketotifen on lipid metabolism

As shown in Fig. 2, when compared with normal control group, both DM and KT groups showed significantly higher levels of TC, TG and LDL and significantly lower levels of HDL before ketotifen treatment (All $p < 0.05$). After four weeks of ketotifen treatment, concentrations of TC, TG and LDL in KT group decreased significantly and HDL level increased significantly when compared with DM group (All $p < 0.05$). Also, levels of TC, TG and LDL in KT group decreased further and HDL level increased after eight weeks of ketotifen treatment (All $p < 0.05$).

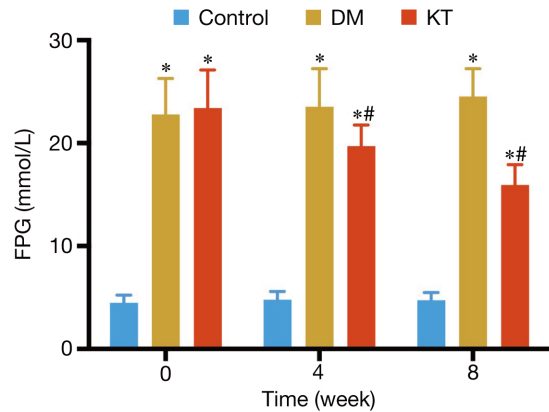


Fig. 1 Effect of ketotifen on fasting plasma glucose concentration. Data are presented as mean \pm SD; * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the DM group. Abbreviation: FPG, fasting plasma glucose; DM, diabetes; KT, ketotifen.

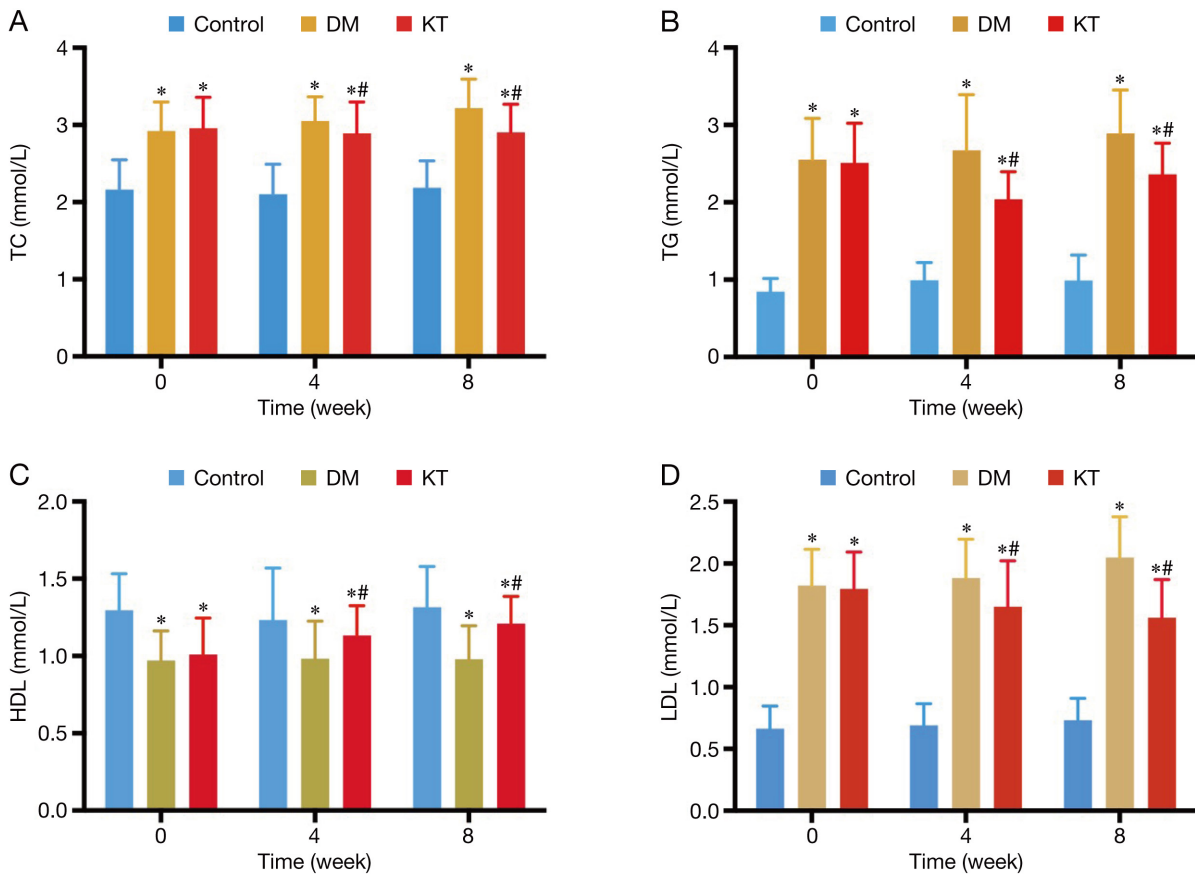


Fig. 2 Effect of ketotifen on lipid metabolism

Data are presented as mean \pm SD; * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the DM group. Abbreviation: TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DM, diabetes; KT, ketotifen.

Effect of ketotifen on levels of IL-6 and TNF- α

Compared with normal control group, both DM and KT groups showed significantly higher levels of IL-6 and TNF- α before ketotifen treatment (Both $p < 0.05$). After four weeks of ketotifen treatment, both IL-6 and TNF- α levels in KT group decreased significantly when compared with DM group (Both $p < 0.05$). Also, IL-6 and TNF- α levels of KT group decreased further after eight weeks of ketotifen treatment (Both $p < 0.05$) (Fig. 3).

Effect of ketotifen on content of malondialdehyde and activity of superoxide dismutase

Compared with normal control group, both DM and KT groups showed significantly higher contents of

MDA before ketotifen treatment (Both $p < 0.05$). After four weeks of ketotifen treatment, the content of MDA in KT group decreased, but there was no significant difference when compared with DM group. While, after eight weeks of ketotifen treatment, the content of MDA in KT group decreased significantly when compared with DM group ($p < 0.05$) (Fig. 4A). Compared with normal control group, both DM and KT groups showed significantly lower activity of SOD before ketotifen treatment (Both $p < 0.05$). After four weeks of ketotifen treatment, the activity of SOD in KT group increased significantly when compared with DM group. Also after eight weeks of ketotifen treatment, the activity of SOD in KT group increased further when compared with DM group ($p < 0.05$) (Fig. 4B).

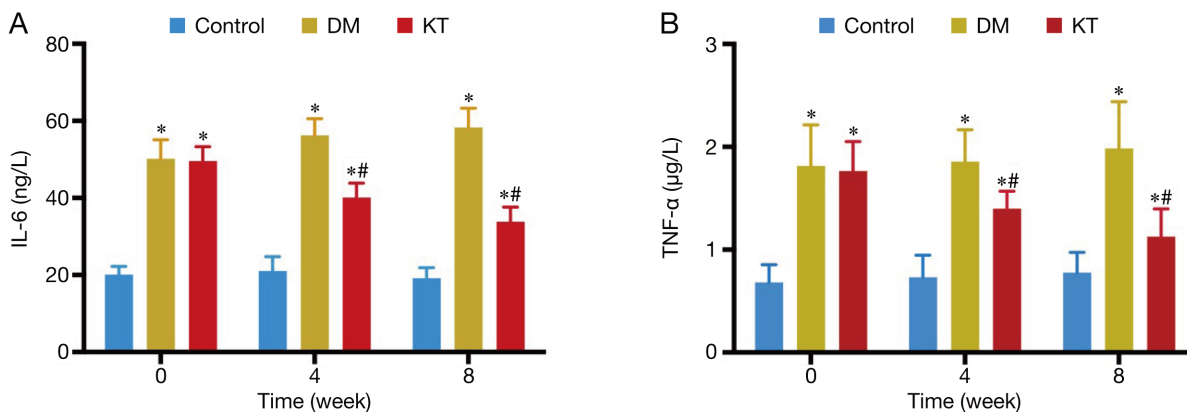


Fig. 3 Effect of ketotifen on levels of IL-6 and TNF- α

Data are presented as mean \pm SD; * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the DM group. Abbreviation: IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; DM, diabetes; KT, ketotifen.

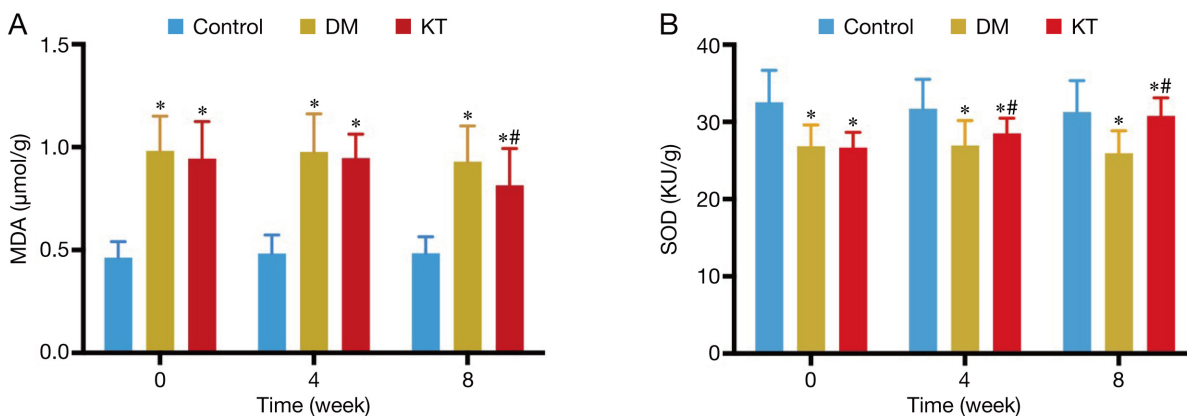


Fig. 4 Effect of ketotifen on content of malondialdehyde and activity of superoxide dismutase

Data are presented as mean \pm SD; * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the DM group. Abbreviation: MDA, malondialdehyde; SOD, superoxide dismutase; DM, diabetes; KT, ketotifen.

Discussion

In the present study, we used ketotifen as a treatment to explore its effects on metabolism and oxidative stress in rats. We found that ketotifen reduced body weight, and improved glucose and lipid metabolism effectively and significantly. And, after ketotifen treatment, the degrees of insulin resistance, inflammation and oxidative stress decreased obviously.

Chronic inflammation and oxidative stress are regarded as pathophysiologic mechanisms of type 2 diabetes, which impair glucose metabolism and increase risks of other metabolic complications [15, 16]. In our study, before treatment, we found indicators of inflammation including IL-6 and TNF- α increased and biomarkers of oxidative stress like MDA and SOD varied significantly in rats model. These results further demonstrated the roles of inflammation and oxidative stress in type 2 diabetes. Therefore, antidiabetic drugs that can alleviate the status of inflammation and oxidative stress might be helpful for diabetes patients.

Generally, oral antidiabetic drugs include sulfonylureas, metformin, thiazolidinediones, benzoic acid derivatives, α -glucosidase inhibitors and others. However, type 2 diabetes is a complex disease for treatment, and there is no specific treatment algorithm that will be appropriate for all patients [17]. Recently, it has been reported that ketotifen as a mast cell stabilizer has a novel medication for obesity and diabetes [14]. Sahar M. *et al.* found that compared with those who only received glimepiride, the co-administration of ketotifen and glimepiride improves glycemic, lipid and inflammatory process in obese patients with type 2 diabetes [13]. In our study, we not only found glycemic, lipid and inflammatory process could be improved by the ketotifen treatment alone, but also found the improvement of hyperinsulinemia, insulin resistance and oxidative stress.

Some explanations may be applied for these effects. Diabetes is in a very close relationship with inflammation and oxidative stress, and both inflammation and oxidative stress impair pancreatic islet β cell function and insulin resistance, which are the key causes of diabetes [18]. Mast cell, one kind of important inflammatory cell, which participates in immune responses during allergic reactions. Recently, it has been suggested that mast cells also play important roles in other inflammatory diseases, inflammatory bowel disease, arthritis, metabolic bone disease, atherosclerosis, obesity and diabetes [14]. After activation, mast cells degranulate

releasing proteases, cytokines, interleukin (IL)-6 and interferon (IFN)- γ [19], and may enhance glycolytic pathway activity and increase electron donor into mitochondrial, which lead to accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and finally become oxidative stress [20, 21].

In this study, improvement of insulin resistance and decreased levels of glucose and inflammatory and oxidative stress indicators after ketotifen intervention, suggesting that ketotifen can improve insulin resistance, and inhibit the process of mast cells to release inflammatory mediators, and may reduce oxidative stress on β cells. And with the extension of the intervention time, these effects were more significant. And along with the improvement of glucose, inflammation and oxidative stress and lipid metabolism in diabetic rats have been significantly improved. So we believe that ketotifen possibly achieve the anti-inflammatory, improvement of insulin resistance and oxidative stress, thereby further improving lipid metabolism disorders through inhibition of mast cell activity.

As far as we know, this was the first study to explore the effects of ketotifen on metabolism, inflammation and oxidative stress. These results will provide with us new strategies for diabetes treatment. However, this study was performed in animal model with a relatively small sample size, therefore prospective clinical trials are needed to validate our results.

In conclusion, our study demonstrated that under the intervention of ketotifen, metabolic profiles of diabetic rats improved significantly, and status of inflammation and oxidative stress ameliorated obviously, suggesting ketotifen could be a new drug for diabetes patients. Well-designed prospective clinical researches are needed to confirm our findings.

Acknowledgement

This study was funded by grants from the Wenzhou Science and Technology Bureau in China (Y200100063), the Natural Science Foundation of Zhejiang Province (LQ12H07003) and the Traditional Chinese Medicine Science and Technology Planning of Zhejiang Province (2012ZB104).

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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